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Substrate Incorporation into Lipids and Proteins in Human Liver Slices

An experimental study with special reference to dyslipoproteinemic conditions

By Håkan Stakeberg

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By

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This thesis is based on the following papers

I Stakeberg H. Gustafson A. & Scherstén T Incorporation rate of leucine into proteins in human liver slices Submitted for publication in Europ J Clin Invest

II Stakeberg H Lundborg H & Scherstén T Incorporation rate <u>in vitro</u> of precursors into hepatic lipids and proteins in patients with extrahepatic cholestasis Submitted for publication in Europ J Clin Invest

III Stakeberg H. & Scherstén T Substrate incorporation into hepatic lipids and proteins <u>in vitro</u> in patients with prebeta hyperlipoproteinemia Submitted for publication in Scand. J Clin Lab Invest

IV Cahlin E Jönsson Jane Persson B Stakeberg H Björntorp P Gustafson A. & Scherstén T Sucrose feeding in man Effects on substrate incorporation into hepatic triglycerides and phosphoglycerides <u>in vitro</u> and on removal of intravenous fat in patients with hyperlipoproteinemia Scand J Clin Lab Invest 32 21 1973

These papers will be referred to by their Roman numbers

CONTENTS

In 1950 J W Gofman (35) suggested that coronary heart disease (CHD) was associated with high plasma levels of certain lipoprotein density classes Since then retrospective (17 18 22 27 40) as well as prospective studies (12) have disclosed that several types of hyperlipoproteinemia are risk factors in the development of CHD (for reviews cf 93 13)

A great deal of work has been done to classify these hyperlipoproteinemic conditions and to elucidate their etiological mechanisms (for reviews cf 32 56)

Generally speaking an increased concentration of circulating lipoproteins may be the result of an increased production rate a decreased removal rate or a combination of both The liver is the primary site of the synthesis and secretion of low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) (46) The removal of VLDL on the other hand, is at least partly dependent on the activity of adipose tissue lipoprotein lipase (75) It is now accepted that in patients with endogenous hyperlipoproteinemia the VLDL concentration (often referred to as prebeta lipoproteins) in the plasma may be increased and maintained after sucrose feeding (for reviews cf IV) Most information on the effect of dietary carbohydrates on the lipid metabolism however is derived from studies in animals Furthermore the kinetic behavior of plasma and liver lipids has in many instances indicated marked species differences (2 28 39 57) and many results obtained from animal studies cannot be extrapolated directly to man.

Most investigations in human beings on the mechanisms of dyslipoproteinemic conditions have applied a kinetic approach Thus either isotope-labeled glycerol or fatty acids have been administered intravenously as precursors for the endogenous hepatic lipoprotein synthesis and secretion into the blood after which the decay of lipoprotein radioactivity has been followed The results of these studies have been conflicting however partly because of methodological problems This has recently been critically reviewed by Nikkilä et al (55 56)

So far a great deal of knowledge has been gained about the production and removal of plasma lipids On the other hand only sparse information with respect to the metabolism of the protein part of the lipoprotein is available It has been suggested that the synthesis rate of carrier proteins (apoproteins) may be of importance for at least the VLDL secretion rate from the liver (14 32 46 62)

This study was designed to obtain more direct information regarding the contributive role of the liver in the genesis of different dyslipoproteine-

mic conditions in man

Extrahepatic cholestasis in man is associated with well-defined changes in the plasma lipoprotein pattern (33 69 75 76 77 83 90) These changes are suggested to be at least partly secondary to a changed hepatic lipid- and protein metabolism as indicated by studies in animals (47 73 92) and in man (86) Therefore patients with this disorder were studied and served as models for a dyslipoproteinemic condition with a liver metabolic involvement The main purpose of this investigation however was to characterize and evaluate the role of the hepatic protein and lipid synthesis rate in the production of increased plasma concentrations of VLDL in patients with prebeta hyperlipoproteinemia

An in vitro technique with human liver slices to determine the triglyceride and phosphoglyceride synthesis capacity has previously been described by Nilsson & Scherstén (58) from this laboratory Another aim of this study therefore was to work out and characterize a method to measure the capacity of human liver slices to incorporate amino acids into proteins as an expression of the protein synthesis rate

Anesthesia and liver biopsy

The patients were premedicated one hour before the introduction of general anesthesia by means of diazepam and atropine Anesthesia was given as hexobarbital (Evipan[R]) nitrous oxide oxygen and succinyl choline Liver tissue for in vitro studies was obtained by a biopsy technique according to Björntorp et al (9) The technique was described in detail by Gottfries (36) The biopsies were performed immediately after the abdominal cavity was opened and the excised liver tissue was placed in ice-cold buffer solution and rapid ly brought to the laboratory (<= 5 min.)

Determination of the incorporation rate of leucine into proteins in human liver slices (I II III)

Different preparations to determine the amino acid incorporation rate in vitro into hepatic proteins in experimental animals e g subcellular sys tems (44 61) suspensions of free cells (82) liver slices (20 21 68) and perfused liver (45 53) have been described As a very complicated sequence of reactions such as the protein synthesis is studied it is desirable to preserve the integrity of the liver tissue as much as possible For obvious reasons the organ preparation representing the highest degree of integrity the perfused liver could not be chosen for studies in man Moreover the tissue slice technique gives if not quantitatively so at least semiquantitatively the same information provided that the basic conditions with respect to the influence of the substrate composition and concentration are well characterized (68)

Using the technique described in detail in Paper I it was possible to determine with sufficient accuracy the incorporation rate of leucine into soluble proteins in human liver tissue slices It was demonstrated that zero order kinetics were obtained for at least four hours when 500 mg liver slices were incubated in a "complete amino acid" medium The incorporation rate of leucine into proteins revealed saturation kinetics at a concentration four times that of the serum of a complete amino acid mixture in the incubation medium. In this system we found an intracellular leucine concentration of the same magnitude as in the medium (based on calculations of determined inulin space and diffusible water) According to Peters et al (68) and Mortimore et al (53) as reviewed in Paper I the specific activity of the precursor amino acid in the medium may be valid for the calculation of the protein synthesis rate under these conditions

Addition of insulin to the medium (100 mU/ml) increased the incor poration rate of leucine by 32 % in three consecutive experiments whereas

supplementation with glucose (5 5 mmol/l) or fructose (5 5 mmol/l) caused no change or an inhibition of the incorporation rate of leucine respectively These findings are in good agreement with previous studies in experimental animals (11 51 54) and indicate that the technique described is adequate to reveal changes in the incorporation rate of leucine into liver proteins in man at least as accurately as previously described for experimental animal liver tissue preparations

Leucine is metabolized in the liver to a low extent Thus label was found in CO_2 aceto-acetate and in lipids This incorporation into various metabolites represented a small fraction however which agrees with other reports (26 68) This finding probably reflect the relative inability of the liver to degrade branched-chained amino acids which in turn makes them suitable as labeled precursors in in vitro studies of the present kind

The standard error of the method for the incorporation rate of leucine into proteins calculated on the basis of 38 duplicates was _ 9 5 per cent and for the isolation of proteins from liver tissue _ 4 per cent

Determination of the incorporation rates of glycerol and fructose into glycerides and into glycogen in human liver slices (II III IV)

The technique used for this purpose was worked out in this laboratory several years ago (58) For details concerning the incubation procedure see Paper IV

The basic incubation medium consisted of 3 per cent albumin in Krebs Ringer phosphate buffer pH 7 4 Palmitic acid glycerol and fructose were added to this medium to obtain the appropriate concentration of these substances in each individual experiment As radioactive precursors for the lipid synthesis glycerol 1 3-^{14}C or fructose-U-^{14}C were used The incubation time was four hours The incorporation time curve for glycerol and fructose into phospholipids and triglycerides was linear for at least five hours (58) After incubation the liver slices were submerged to 10 ml chloroform-methanol (2/1 v/v) After 22 hours the extract was washed with 10 ml of 0 05 M KCl by a modification of the method of Folch et al (30)

The standard error for the incorporation rate of glycerol into lipids was determined on the basis of a series of duplicates and was found to be _ 9 per cent

The lipid synthesis was calculated from the specific activity of the precursor in the medium and recovered radioactivity in the glycerides and expressed as nmol incorporated glycerol or fructose per gram liver per hour

Analytical procedures

Separation of different lipids in the chloroform-methanol extracts from plasma and liver tissue was performed by thin-layer chromatography (TLC)

according to Gloster & Fletcher (34) The determination of lipid phosphorus was performed according to the Bartlett method (5) as modified by Svennerholm & Vanier (89) (standard error of the method 5 0 per cent) Glyceride-glycerol was determined as described by Carlson (19) (error 3 3 per cent) cholesterol according to Sperry & Webb (85) (error 10 4 per cent) glucose according to Levin et al (50) and glycogen according to van der Vies (94) (error 4 2 per cent) IRI insulin was determined radio-immunochemically according to Hales & Randle (41) using assay kits from Amersham (insulin immuno assay kit the Radiochemical Center Amersham, Buckingham England) (error 7 1 per cent) Protein was quantitated according to Lowry et al (49) (error per cent)

The radioactivity was determined in a Packard Tri-Carb (3320) liquid scintillation spectrometer Correction for quenching was performed by the external standard method.

Experimental procedures in patients on a carbohydrate-enriched diet (III IV)

All patients on a carbohydrate-enriched diet were hospitalized for 20 days and the patient group designated as non-sucrose-fed controls for two days prior to the gallbladder operation In the morning on the day after admission after an overnight fast capillary blood and heparinized venous blood samples were drawn for subsequent determination of blood glucose (50) basal plasma insulin (41) plasma cholesterol (85) and triglycerides (19) and for lipoprotein electrophoresis (71) A glucose tolerance test was performed the same day as described in detail in Paper IV

In the morning of the second day the intravenous fat (Intralipid®) tolerance test (10) was performed with the patient in the fasting state Just before the tolerance test a percutaneous biopsy of adipose tissue was taken from the gluteal region (66) The samples obtained were used to determine the lipoprotein lipase activity (6)

The patients were given 200 g sucrose per day from the third day through the 17 th day The ordinary hospital diet contained an average of 2500 Kcal distributed as 500 Kcal of protein 900 Kcal of fat and 1100 Kcal of carbohydrates mainly as starch With the aid of a dietician approximately 800 Kcal (160 Kcal protein 290 Kcal fat and 350 Kcal carbohydrates) of the daily food intake was replaced by 200 g of sucrose The sucrose solution kindly supplied by the Beecham Company London England was flavored and dispensed in 100 g bottles

Throughout the sucrose feeding period blood samples were taken every second day to determine plasma cholesterol and triglycerides After the sucrose feeding period but before the gallbladder operation a second glucose tolerance test was performed on the 17 th day The fat tolerance test with a concomitant determination of the adipose tissue lipoprotein lipase activity

were repeated on the 18 th day These determinations were performed under the same conditions as before the sucrose feeding period

Statistical methods (I II III IV)

In the comparison between different groups Student s t test was used For pair differences Wilcoxon s test as described by Siegel (84) was used Linear regressions were calculated according to the method of least squares Standard procedures were also used to calculate correlation coefficients T tests were applied to check the significance of regression coefficients Standard error of the methods was calculated from duplicates using the following formulae

$$SE \sqrt{\frac{\sum d^2_i}{n}}$$

where d_i is the difference between duplicate measurements The error was then expressed as a percentage of the mean of all determinations

CLINICAL MATERIAL

The clinical material consisted of 105 subjects 38 men and 67 women aged 20-86 years All patients except those with extrahepatic cholestasis (II) had a normal liver function according to the following criteria serum bilirubin $<$ 1 0 mg/100 ml thymol turbidity $<$ 0 10 alkaline phosphatase (EC 3 1 3 1) $<$ 10 units (Buch) L aspartate 2-oxoglutarate (EC 2 6 1 1) $<$ 20 units ($NAD/NADH_2$)

None of the patients had any known disease apart from the gallbladder disease and in pertinent cases prebeta hyperlipoproteinemia (except Patient No 1 Paper III who had slight maturity onset diabetes mellitus treated with sulfonylurea) At the time of the study or shortly before none was taking any drug known to affect the liver function Patients with known or suspected alcoholic problems and women taking oral contraceptive steroids were excluded from the study

The patients were divided into different groups on the basis of their initial plasma lipoprotein pattern according to Fredrickson & Lees (6 31) The plasma lipoprotein type was determined from repeated plasma lipid analyses lipoprotein electrophoresis on agarose gel (71) and alphalipoprotein cholesterol determinations (6) Based on these analyses determinations of prebeta lipoprotein and beta lipoprotein cholesterol were performed according to Gustafson et al (40) The lipoprotein values were used for the subsequent lipoprotein typing The presence at repeated analyses of prebeta lipoproteins at agarose gel electrophoresis concomitant with $>$ 35 mg/100 ml of prebeta lipoprotein cholesterol were used as definition of prebeta hyperlipoproteinemia The corresponding upper limit for beta lipoprotein cholesterol was set at 200-220 mg/100 ml dependent on the patient s age and sex

The group with prebeta hyperlipoproteinemia (n 21) comprised 6 patients with hyperlipoproteinemia Type II B and 15 patients with Type IV All controls were admitted to the hospital for an operation for uncomplicated gallbladder disease The plasma triglyceride and cholesterol concentrations in the control group were not different from those of middle-aged men and women randomly selected from the same city (8)

Fifteen of the patients studied had extrahepatic cholestasis (II) Clinical data on the time of the liver biopsy and the blood sampling are reported in detail in Paper II In all patients the diagnosis was confirmed at laparotomy and in the five tumor patients by means of microscopy No detectable metastasis in lymph nodes or liver was found in any of these patients Patients Nos 1 3 and 11 had localized carcinoma of the head of the pancreas strangulating the common bile duct while patients Nos 2 and 14 had small

isolated bile duct carcinomas

Consent was obtained from all patients on the carbohydrate-enriched dietary regimen No complications in connection with the liver biopsies or the other procedures were encountered

Incorporation rate of leucine into hepatic proteins in vitro in normolipoproteinemic controls (I)

The incorporation rate of leucine into proteins in liver slices from 42 normolipidemic gallstone patients was studied with the technique described in detail in Paper I No sex differences were found In subjects older than 60 years we found a higher ($P < 0\ 025$) incorporation rate of leucine into proteins than in subjects below the age of 60 Since the protein content in the liver tissue from these subjects was approximately the same as in the younger ones this finding may be interpreted as a more rapid hepatic protein turnover rate in older subjects This would be in line with the high lysosomal enzyme activity in aging human liver tissue (7) and is in agreement with the finding of an increased synthesis rate of albumin <u>in vivo</u> in the old rat (64)

Provided the incorporation rate of leucine into liver proteins can be taken as an expression of protein synthesis it is possible to calculate the synthesis rate by dividing the amount of leucine incorporated into hepatic proteins by the mass fraction of leucine in liver proteins 0 09 (w/w) (52) This will give a synthesis rate of 0 100 mg protein per hour per gram liver tissue which is roughly 1/10 of the corresponding synthesis rate for rat liver <u>in vitro</u> (68) Our results might indicate a protein turnover corresponding to the half life of soluble liver proteins of 3. days in man It has to be emphasized however that the protein synthesis rate determined <u>in vitro</u> in rat liver slices has been found to be 1/5 1/10 of the rate determined <u>in vivo</u> (68)

It might be questioned whether the liver tissue used in the protein synthesis experiments can be considered normal Sunzel et al (87) reported a higher triglyceride content (but no differences of phospholipids and cholesterol) in liver tissue from patients with gallstone disease than in patients with peptic ulcer Nilsson & Scherstén (58) however found no differences in the lipid synthesis pattern in liver slices from patients with diseases as different as gallstone disease gastric disease and renal calculus So far this seems to hold true also for the protein synthesis This supports the assumption that the gallstone disease of the patients in the present study did not influence the results especially not the comparison between the different patient groups

Substrate incorporation into hepatic lipids and proteins in vitro in extra hepatic cholestasis (II)

In liver tissue from fifteen patients with extrahepatic cholestasis we found an increased concentration ($P < 0\ 025$) of phosphoglycerides and pro-

teins compared with normolipidemic gallstone patients The triglyceride concentration was equal while the glycogen concentration was decreased ($P < 0.05$) in these patients The changed lipid and protein concentrations could not be ascribed to a change in the hepatic water content which was equal in the two groups

The incorporation rate of glycerol into total phosphoglycerides ($P < 0.01$) as well as into the choline phosphoglycerides ($P < 0.05$) and ethanol amine phosphoglycerides ($P < 0.01$) was increased in cholestatic liver tissue compared with controls On the other hand liver tissue from patients with cholestasis incorporated glycerol into triglycerides at the same rate as controls

The incorporation rate of leucine into liver proteins was increased ($P < 0.01.5$) in the cholestatic liver tissue which also had a high protein content ($P < 0.025$) compared with the controls These findings may be interpreted as indicating an enhanced synthesis of phosphoglycerides and proteins in cholestatic liver tissue

This interpretation was supported by the higher concentrations of phosphoglycerides and proteins in the cholestatic liver slices The final interpretation of these data should be made with caution however since the possibility of a changed specific activity in the immediate precursor pool can not be ruled out completely The fact that the incorporation rate of glycerol into triglycerides was unchanged concomitant with a significant increase in the incorporation rate into phosphoglycerides does not support the hypothesis of a changed specific activity in the α glycerophosphate pool Moreover the free leucine concentration was found to be almost equal (600-700 µmol/l) in cholestatic and normal liver tissue

An enhanced synthesis of phosphoglycerides in liver tissue under cholestatic conditions may be explained by an increased bile acid concentration in the liver Greim et al (38) have shown that biliary obstruction causes an increased cholic acid concentration comparatively early whereas chenodeoxycholic acid increased only when the cholestasis was protracted A stimulating effect of bile acids on the lecithin synthesis seems to be well established in different species (3 42 59 79 81 88) The mechanism of the effect of bile acids on the phospholipid synthesis is not known however we have previously shown (15) that in vitro addition of bile acids to incubations with liver slices caused a shunting of glycerol into lecithin synthesis pathways This finding provided support for the hypothesis that bile acids in some way directly affect the hepatic synthesis of lecithin This hypothesis is also supported by the finding of Kennedy & Weiss (48) that the lecithin synthesis in rat liver preparations was stimulated in the presence of detergents such as bile acids

The mechanism behind the increased protein synthesis in cholestatic liver tissue is not easy to explain An increased incorporation rate of amino acids into hepatic proteins in experimentally induced cholestasis in rats has been found (73) Morphometric analyses of electron micrographs of liver tissue from patients with cholestasis have shown an increased surface area of both the rough and smooth endoplasmic reticulum (37) This probably indicates a 'hypertrophy' in that part of the cell which is mainly responsible for the protein synthesis

Our finding of a significant correlation between the incorporation rate of glycerol into phosphoglycerides and the incorporation rate of leucine into proteins in cholestatic liver may suggest a common mechanism for the enhancement of both these processes In one series of experiments with control liver slices however glycocholic acid or glycochenodeoxycholic acid was added to the incubation medium (final concentration 0 05 mmol/l) Neither one of these primary bile acids influenced the incorporation rate of leucine into proteins in contrast to the effect of the bile acids on the incorporation rate of glycerol into phosphoglycerides (79)

Another contributive mechanism to the enhanced incorporation of glycerol into fatty acids and leucine into proteins in cholestatic liver tissue has to be considered. Thus the reduced form of nicotin-amide-adenine nucleotide monophosphate has been found to enhance the synthesis of amino acids and proteins (95) In a previous work Scherstén et al (78 80) found that the ratio between reduced and oxidized nucleotides was considerably increased in cholestatic liver tissue Thus their findings are in line with those of the present study

As pointed out in the introduction extrahepatic cholestasis can be looked upon as a model system for dyslipoproteinemic conditions Whether the changed plasma lipoprotein pattern in cholestasis is related to the changed liver tissue metabolism can not be unequivocally determined on the basis of the present results It appears tempting however to assume that the increased plasma lecithin concentration in this condition may be caused at least partly by the enhanced hepatic synthesis An additional cause may be a diminished lecithin secretion into bile The appearance of the abnormal lipoprotein-X may be associated with such changes in the protein and phospholipid synthesis in the liver

Effects of sucrose feeding in patients with prebeta hyperlipoproteinemia (III, IV)

Four patient groups were studied as described in detail in Papers III and IV They are referred to as non-sucrose-fed controls (n 36) non sucrose-fed patients with prebeta hyperlipoproteinemia (n 8) sucrose-fed controls

(n 15) and sucrose-fed patients with prebeta hyperlipoproteinemia (n 13)

In the normolipoproteinemic patients sucrose feeding produced an increase in the plasma triglycerides (P ≤ 0 001) a transient decrease in the plasma cholesterol (P ≤ 0 005) an increase in basal insulin (P ≤ 0 01) concomitant with an increased lipoprotein lipase activity (P ≤ 0 01) Only basal insulin increased significantly in the sucrose-fed patients with prebeta hyperlipoproteinemia

Compared with non-sucrose-fed controls the liver triglyceride content was increased in sucrose-fed controls (P ≤ 0 05) as well as in non-sucrose-fed (P ≤ 0 05) and sucrose-fed patients with prebeta hyperlipoproteinemia (P ≤ 0 05) An increased incorporation rate of glycerol into hepatic triglycerides was found in the above-mentioned groups except for the sucrose-fed patients with prebeta hyperlipoproteinemia The higher incorporation rate of precursors into triglycerides was taken as an indication of an increased hepatic triglyceride synthesis in the sucrose-fed controls and the non-sucrose-fed patients with prebeta hyperlipoproteinemia. The increased liver triglyceride content supports the validity of this finding An additional fact which supported the finding was the significant correlation between the incorporation rate of precursors into hepatic triglycerides and the increment of plasma triglycerides on the seventh day of sucrose feeding in the sucrose-fed controls as well as the significant correlation between the hepatic triglyceride synthesis and the plasma triglyceride concentration in the non-sucrose-fed patients with prebeta hyperlipoproteinemia

In the sucrose-fed patients with prebeta hyperlipoproteinemia the mean plasma triglycerides as well as the mean incorporation rate of precursors into hepatic triglycerides showed a wide range of values This may be an expression of heterogeneity within this series of biochemically typed patients with prebeta hyperlipoproteinemia It should be kept in mind that this patient group in addition to comprising patients with Type IV also included 6 patients with Type II B Furthermore it remains to be shown that patients with Type IV hyperlipoproteinemia constitute a metabolically homogenous population (70)

In the sucrose-fed patients with prebeta hyperlipoproteinemia the mean of the incorporation rate of radioactive precursors into triglycerides was moderately increased but less than in the sucrose-fed controls Caution should be exercised in the final interpretation of these data however since the possibility of a dilution of the label by an expanded precursor pool of α-glycerophosphate can not be completely ruled out The facts that the incorporation rates of glycerol and fructose into triglycerides showed an increase together with a highly significant decrease in the incorporation rate into phosphoglycerides however do not support the hypothesis of a dilution of the

immediate precursor pool Furthermore these findings are in agreement with conclusions drawn from other investigations in man (24 29 70)

The finding of a lower incorporation rate of glycerol as well as of fructose into hepatic choline phosphoglycerides in the sucrose-fed patients with prebeta hyperlipoproteinemia seems to be further supported by the lower liver content of phosphoglycerides in these patients These data are also in agreement with our observation of a low choline phosphoglyceride concentration in the bile from the same patient group (16) The data also concur with the recent finding of Park et al (65) that in high carbohydrate-fed rats the choline phosphoglyceride level in the rough microsome fraction of liver homogenates was significantly decreased and that the linoleic and arachidonic acids were virtually absent in the phospholipids of this fraction Recently Alling et al (1) showed that sucrose feeding rapidly caused a significant decrease in the proportion of linoleic acid in liver plasma and bile choline phosphoglycerides indicating that sucrose feeding inhibits the cytidindiphosphate choline pathway (Kennedy's pathway) of the choline phosphoglyceride synthesis

Thus this decreased choline phosphoglyceride synthesis seems to be provoked by sucrose feeding since non-sucrose-fed patients with prebeta hyperlipoproteinemia had a normal incorporation rate of precursors into hepatic glycerides as well as a normal hepatic phosphoglyceride content Prebeta hyperlipoproteinemia is not characterized by low plasma phosphoglycerides or plasma choline phosphoglycerides (60) but the VLDL in Type IV have a lower specific density and a higher Sf value (4) and would therefore have a higher triglyceride/phosphoglyceride ratio than that of normolipoproteinemic subjects

The low incorporation rates of glycerol and fructose into phosphoglycerides are most likely not an effect of the conditions chosen for the *in vitro* incubation Neither an increase in the fatty acid amount in the system (58 63) nor the use of another fatty acid substrate like oleic acid (43) or linoleic acid (15) would be expected to increase the incorporation into phosphoglycerides The addition of insulin to the system would be expected to influence the incorporation into phosphoglycerides only to a moderate extent (91)

The incorporation rate of leucine into hepatic proteins was equal in non-sucrose-fed controls (n 20) and non-sucrose-fed patients with prebeta hyperlipoproteinemia (n 9) Three of the sucrose-fed controls (n 4) incorporated leucine into proteins at a rate above the 95 per cent confidence level of that for non-sucrose-fed controls This may indicate an insulin effect on the hepatic protein synthesis capacity (I 51) which has been found also after long-term glucose feeding in rats (23) No change in the incorporation rate of leucine into hepatic proteins in sucrose-fed patients with prebeta

(n = 15) and sucrose-fed patients with prebeta hyperlipoproteinemia (n = 13)

In the normolipoproteinemic patients sucrose feeding produced an increase in the plasma triglycerides ($P < 0.001$) a transient decrease in the plasma cholesterol ($P < 0.005$) an increase in basal insulin ($P < 0.01$) concomitant with an increased lipoprotein lipase activity ($P < 0.01$) Only basal insulin increased significantly in the sucrose-fed patients with prebeta hyperlipoproteinemia.

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SUMMARY

1 Liver tissue from normolipidemic gallstone patients older than 60 years incorporated leucine into hepatic proteins at a higher rate than liver tissue from younger individuals indicating a higher protein turnover rate in the older patients

2 Cholestatic liver tissue contained more proteins and phosphoglycerides and incorporated precursors into proteins and phosphoglycerides at a higher rate than presumably normal liver tissue The incorporation rate of glycerol into hepatic phosphoglycerides correlated significantly with the plasma phosphoglyceride concentration

3 Liver tissue from patients with prebeta hyperlipoproteinemia on an ordinary diet contained more triglycerides and incorporated glycerol into triglycerides at a higher rate than liver tissue from normolipidemic gall stone patients The incorporation rate of precursors into hepatic triglycerides correlated significantly with the plasma triglyceride concentration indicating an increased hepatic triglyceride synthesis rate

4 *After sucrose feeding for 14 days liver tissue from patients with pre*beta hyperlipoproteinemia had a lower content of phosphoglycerides and incorporated precursors into phosphoglycerides at a lower rate compared with non-sucrose-fed patients with prebeta hyperlipoproteinemia indicating a phosphoglyceride synthesis defect provoked by the sucrose feeding

5 Sucrose-fed controls showed an increased incorporation rate of glycerol and fructose into hepatic triglycerides in vitro which correlated with the increase in the plasma triglyceride concentration indicating an increased triglyceride synthesis rate

6 The fractional elimination rate of exogenous fat decreased after sucrose feeding in controls as well as in patients with *prebeta hyperlipoproteinemia* indicating a removal defect for at least exogenous fat

7 The adipose tissue lipoprotein lipase activity was increased after sucrose feeding in controls and was inversely correlated to the plasma triglyceride concentration in controls as well as in patients with prebeta hyperlipoproteinemia.

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Hepatic Circulation in Hepatitis A

A Study in Young Males at Rest and during and after Supine Leg Exercise

By Per Lundbergh

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HEPATIC CIRCULATION IN HEPATITIS A

A Study in Young Males at Rest and during and after Supine Leg Exercise

By
PER LUNDBERGH

STOCKHOLM 1974

To Inger
Fredrik and Elisabet

This thesis is based on the following papers

I Lundbergh P & Strandell T : The effect of physical exercise on the wedged and free hepatic venous pressure in normal men Acta med scand 194:211 1973

II Lundbergh P & Strandell T Changes in hepatic circulation at rest during and after exercise in young mal with infectious hepatitis compared with controls Acta med scand Preprint

III Lundbergh P & Strandell T : Hepatic wash-out curves of ^{85}Kr and ^{133}Xe after retrograde hepatic venous inje tions in pati nts with infectious hepatitis and control Accepted for publication in Scand J clin Lab Invest

IV Lundbergh P : Hepatic circulation during and after infectiou hepatitis Accepted for publication in Scand J Infect Dis

They will be referred to by the Roman numerals I-IV

CONTENTS

INTRODUCTION

About 2000 case f viral hepatitis are reported in Sweden every year one-third of them being infectious hepatitis or viral hepatiti type A and two-thirds serum hepatiti or viral hepatitis type B In ccordance with WHO techni al report (1973) the terms hepatitis A and hepatitis B will be used in this thesis

Patients with viral hepatitis are ani teri or icteric and the disease is acute subacute or chronic The acute i teri form of hepatiti A i characterised by a pre-icteric stage with symptoms such as lassitude loss of ppetite fever nause vomiting headache abdominal discomf rt myalgia arthralgi and exanthema Within a week jaundice i noted On physical examination the typical patient has a tender and enlarged liver but the hepatomegalia i rarely massive The splee i palpable in about 20 % f the patients (She lock 1968) The symptoms subside as jaundice fade and in adults the icteric stage last f r an average f 3-4 weeks

The histopathologi change seen at light mi roscopy f liver specimens from pati nts with cute hepatiti have been described by Roholm & Iversen (1939) and by Luckó (1944) It i usually beli ved that the picture is ide tical in hepatiti A and B but mino diff rence have been bserved (Iwarso t al 1972) The change characteristi of both forms are single cell degeneration focal necrosi cellular infiltration f predominantly mononuclear cells specially in the enlarged and edematous portal tracts and reticuloendothelial prolif rati n fte with cells protruding into the hepatic sinusoids Regeneration proceeds together with necrosi the cell adj ining the central vein are usually the last to recover (Sherlock 1968) and on an verage normal architecture is restored within 2-3 months (Wepl & Wildhirt 1968)

At el tron microscopy the following hepati change have also been seen The profil f the rough ndoplasmic reticulum are dilated (Jézéquel & Steiner 1967 Teodori t al 1967) sinusoidal microvilli are decreased in numbe and those present are fte swoll (Teodori & Gentilini 1967); the hepatocytes are separated from ach other (Huebner & Slusser 1968); the space f Disse i widened and filled with amorphous debri and collagen fibrils (Teodori & Gentilini 1967); and basement membrane are present bene th the endothelial lining (Schaffne 1966)

During and after the seco d world war the importance of strict bed rest and high protein intake in the treatment of patients with viral hepatiti was stressed (Barker et al 1945 Hughes 1945) The importance of bed-rest in the healing of viral hepatitis was further emphasized by the findings in healthy subjects that hepatic blood flow decreased significantly on changes from supine to upright position (Culbertson et al 1947) as well as during exercise (Bradley 1949)

This strict bed rest regimen was soon questioned (Klatskin & Rappaport 1947 Gardner et al 1949 Swift et al 1950 Nelson et al 1954) The great challenge was made by Chalmer et al (1955) who in th ir thourough Korean study concluded: Pati nts hospitalized with acute inf ctious hepatitis improved as rapidly when allowed to be up and about their wards at will as did patients kept at strictly enforced bed rest In a 10-year follow-up study by Nefager & Chalmers (1963) no difference f significance wa found between those treated with strict and those treated with ad libitum bed-rest The opinio in the late 1960s a stated in curre t textbooks (Sherlock 1968 Mosley & Galambos 1969) was that regardle s of the depth of jaundice patients who had normal appetite and felt well may ambulate in th ward within the limits of physical fatigue

To conclude patients with hepatitis A have symptoms and signs of impaired hepatic function that might be attributabl to a reduced number f hepatocyte and an insufficiency of those surviving The protrusion of cells into the sinusoids changes of the sinusoidal surface widening f Disse s space and presence of basement membranes might affect local hepati blood flow and the exchange between liver and blood thus contributing to the impaired liver function

The object of the present investigation was to find out to what extent these morphologic changes of the live in hepatitis A are ttended with measurable changes in hepati circulation by

1) analysing the hepatic circulation at rest and
2) studying the ffect of xercise on hepatic circulation and finding out whether the chose physical exercise provoked any signs of impaired liver fun tion

MATERIAL

Males between 20 and 30 years old with no history of drug or alcohol abuse treated for acute i terio hepatitis-B-antigen-(HBAg)-negative viral hepatiti at the Roslagstull Hospital Stockholm over the period April 1972 - Octobe 1973 were asked to participate in this study and 10 out of 14 pati nts all af brile consented Although being sporadic the cases were diagnosed s hepatiti A since clinical and laboratory findings were not consiste t with inf ction due to Epstein-Barr virus or to cytomegalovirus the onset of the disease was acute and the patient were HBAg-negative a early s 5 (2-8) days after onset f jaundice (II) Theref re according to hospital routine liver bi psy was not performed The results of the bi chemical liver tests on the day f the catheterizati n are shown in Table I and their maximal abn rmalities elsewhere (II) None f the patients had a previ history f liver disease and they were therwise in good health Patients no 3 6 and 7 were well-trained and the others rdinarily trained except patient no 10 who was untrained

After clinical examination 17 healthy male volunteers f the same age were studi d a control group None f them had previous history f liver heart lung or kidney disease and all denied drug and alcohol buse Routine urine and blood test in luding standard bi chemical liver test (Table I) showed no abnormalities and the subject were all HBAg-negative Controls no 4 5 and 10 were well-trained the there rdinarily trained

The total material onsisted f 27 subj t (Table II) Three patients were re-examined but all the subje t did not participate in all the studi The first study (I) comprised 10 controls nos 2 3 4 15 5 16 12 17 6 and 13; the se ond study (II) 10 patient no 1 10 and 13 controls nos 1-13; the third study (III) 8 patients nos 1 2 3 5 6 7 8 and 9 and 10 co trols nos 1 4 5 7 8 9 12 13 14 and 15; and the fourth study (IV) 3 pati ts nos 7 8 a d 10

There was diff rence between the group f patients and the control group with respect t ge body si blood volume hemoglobin and hematocrit (Table II)

All subjects were studied in the morning without premedication Because

of the risk of hypoglycemia in the patients during the 4-hour study including a 40-minute period of exercise the initial subjects were allowed a light breakfast 2-3 hours before the flow study As there were no differences in blood-glucose values between patients and controls patients nos 7 8 9 and 10 and controls nos 8 9 10 and 11 were studied after overnight fast By group comparison however no pertinent diff rence were found between fasting and non-fasting subject

T bl I Clinical and laboratory data on the day of the hepatic venous catheterization in 10 pati nt and corresponding group mean values f r 13 controls SBil = serum bilirubin ALP = alkaline phosphatase LDH = lactic dehydrogenase SGOT = serum glutamic oxaloacetic transaminase SGPT = serum glutamic pyruvi transaminase Thym = thymol turbidity U = units X̄ = group mean value SD = standard deviation xxx = P < 0 001 xx = P < 0 01 and x = P < 0 05 where P is th probability that the difference between patie t and controls w caused by random factors Upper normal limits given below each biochemical liver test

		Day after onset of jaundice	SBil mg % <1 3	ALP U <3 0	LDH U < 225	SGOT U <40	SGPT U <35	Thym U <6
Patient no	1	15	1 6	2 6	152	43	146	9
	2	23	1 8	2 4	184	81	206	11
	3	14	5 8	4 0	199	320	970	6
	4	12	1 8	2 6	145	120	213	2
	5	14	1 4	2 5	126	36	150	2
	6	10	9 6	2 7	339	710	740	13
	7	22	3 5	2 9	132	79	350	4
	8	15	1 1	3 5	170	78	360	14
	9	17	2 6	2 5	134	37	100	12
	10	16	2 1	2 6	141	88	270	-
	X̄	15 8	3 13	2 83	172 2	159 2	350 5	8 1
	SD	4 0	2 66	0 52	63 3	210 4	284 1	4 7
Controls	X̄	-	0 87	1 84	159 7	18 5	16 9	1 4
	SD	-	0 25	0 34	32 4	4 8	6 3	0 7
Diff rence		-	2 26	0 99	12 5	140 7	333 6	6 7
P		-	xxx [a]	xxx	> 0 50	xxx [a]	xxx	xxx

[a] Wilcoxon s non-parametric testing (Snedecor 1956)

Table II Anthropometri and laboratory data at rest (RI) in the patients and the controls BSA = body surface area WI = first work load WII = second work load Hb = hemoglobin Hct = hematocrit BV = blood volume PV = plasma volume RII = t 30 minutes after exercise Figure within parenthese are not included in mean value Othe symbols as in Table I.

	Age yr	Height cm	Weight kg	BSA m^2	Work load kpm/min WI	Work load kpm/min WII	Duration min WII	Hb g% RI	Hct % RI	BV l RI	PV l RI	PV l RII
Patient no 1	29	177	67	1 85	300	600	21 00	11 7	36 1	4.30	2 89	2 86
2	26	181	73	1 95	350	700	16 50	13 3	38 2	4.63	3 02	2 90
3	27	182	67	1.89	250	500	20 75	13 3	40 8	5 76	3 63	3 26
4	25	186	85	2 11	400	800	21 50	15 0	40 5	6 88	4.35	-
5	21	180	62	1 82	400	700	16.25	14.7	41 1	4.62	2 89	2 74
6	22	186	72	1 98	600	1000	15 25	14.6	43 7	5 63	3 39	-
7	25	186	80	2 06	500	1000	20 75	10 9	34 0	6 65	4.59	4.38
8	23	185	75	2 00	350	700	20 75	13 3	42 8	5 37	3 27	3 15
9	25	190	83	2 13	350	700	20 75	12.7	39 2	4.76	3 06	3 93
10	27	169	63	1 73	300	500	14 00	14.1	40 8	4.07	2.56	2.34
$\bar{x}$	25 0	182	72.7	1 95	380	720	18 75	13 3	39 7	5 27	3 36	3 20
SD	2 4	6	8 1	0 13	103	175	2 88	1.3	3 0	0 96	0 65	0 66
Control no 1	26	182	73	1 96	500	1000	21 00	14 2	36 0	4 84	3 26	-
2	25	187	73	2 00	500	1000	17 70	15 5	42.1	5 13	3 17	-
3	23	179	80	2 00	500	1000	13 75	15 6	40 6	5 56	3 51	-
4	24	181	74	1 95	600	1200	21.25	14.4	40 0	6 54	4.16	
5	27	183	79	2 03	600	1200	21 35	14 9	40 0	5 21	3 31	2.90
6	23	185	73	1 98	550	1000	10 00	15 8	47 1	7 00	4 00	3 70
7	23	183	74	1 97	500	800	16 10	13 7	40 0	4.54	2 90	3 06
8	25	177	64	1 82	350	700	21 70	14.3	43 5	4.30	2 60	2 40
9	20	173	67	1 82	350	700	18 25	14.8	41 7	4 36	2 70	2 48
10	29	192	92	2 25	400	1000	20 75	16 9	46 7	5 50	3 16	2 92
11	26	185	74	1 99	500	1000	20 75	12 9	34.0	4.94	3 41	2 96
12	24	187	73	2 00	600	1200	12 00	13 8	37.4	5 69	3 76	3 42
13	27	175	72	1 88	500	1000	14 25	12.3	41 0	4 51	2 83	3 46
14	(26)	(184)	(80)	(2 05)	(400)	(800)	(20 75)	(14.8)	(41.4)	(6 24)	(4 24)	-
15	(21)	(185)	(83)	(2 08)	(450)	(900)	(21.40)	(16 1)	(43 2)	(5 05)	(3 07)	-
16	(20)	(173)	(55)	(1 67)	(350)	(600)	(21.00)	(13 3)	(43 3)	(3 52)	(2 13)	-
17	(26)	(193)	(100)	(2 34)	-	(1000)	(21.00)	(16 0)	(45 9)	(6 72)	(3 91)	-
$\bar{x}$	24.8	182	74.5	1 97	495	985	17 60	14 5	40 8	5 24	3 27	3 03
SD	2 3	5	6 7	0 11	85	168	4 02	1 3	3 7	0 82	0 50	0 44
Diff rence	0 2	0	-1 8	-0 02	-116	-265	1 15	1 2	-1 1	0 03	0 09	0 17
P	>0 80	>0 99	>0 50	>0 60	xx	xx	>0 40	>0 05	>0 40	>0 90	>0 70	>0 50

METHODS

The subjects were studied during a resting period of about 1 hour during two consecutive 20-minute periods of supine leg exercise on an electrodynamically braked bicycle ergomete (Holmgren & Mattsson 1954) and during 30 minutes after exercise The second work load wa generally twice as high a the first on and chosen so as to correspond to the heavi st load the subject was expected to sustain f r 20 minutes In 7 pati nt no 1-7 and 4 controls nos 6 7 12 and 13 a 15-minute rest period was inserted between the two work loads in order to study the wedged and free hepatic venou pressure also after the first load

In most subjects retrograde injections of ^{85}Kr and ^{133}Xe in saline were give in an o cluded hepati vein at rest before exercise to estimate the perfusion coeffi ients (III) No systemati influence of these in-je tion on the hepatic extractions of oxygen and ICG was observed as judged from omparisons in patients nos 8 and 9 and controls nos 8 and 9 between blood sampled before the first injection and 2-4 minutes there-after as well as before and after the subsequent injection

The pati nts were investigated with utmost care spe ial precautions being taken at all the procedures before during and after the examination

Catheterization With the subje t in the supine position a right hepatic vein wa percutaneously catheterized from a cubital vein under TV fluoros-copy and the radiopaque t flon catheter (1 2/2 0 1 5/2 3 mm) was placed as far caudally and peripherally a possible In the wedged position the tip of the theter was generally located 2-4 cm from the lateral chest wall and in the free position about 4 cm further proximal Short teflon catheters (1 1/1 4 mm) were also percutaneously inserted into the brachial artery and a peripheral vein In 2 patie ts nos 8 and 9 and 5 controls no 7 8 9 10 and 13 anothe right hepatic vein was similarly catheterized from the contralat ral arm In 3 pati nt no 3 5 and 6 and 4 controls nos 2 3 4 and 12 a thin polyethyl ne catheter (PE 60 0 76/1 22 mm) was also percutaneously inserted from a cubital vein into the pulmonary artery

Blood-flow measurements Afte an i v bolus injection of about 15 mg of ICG (Cardio-Green ® Hynson Westcott and Dunning Baltimore Md USA)

stabilized with 0 1 % human albumin solution (Cherrick et al 1960) a constant i v infusion f the dye wa given at a rate of about 1 mg/min After an equilibration period of about 30 minute 4-5 paired blood samples from the artery and hepatic vein were collected the arterial sample about 15 seconds pri to the venous one Blood wa drawn t generally 5-minute intervals at rest during the work loads and the pause if any between them, and after exercise (Fig 1) The plasma content of ICG was measured at 805 nm with individual calibration curves on a Hitachi 101 spe tro-photometer (Hitachi Ltd Tokyo Japan) after correction for plasma turbidity at 900 nm (Nielsen 1963) The reproducibility was studied by

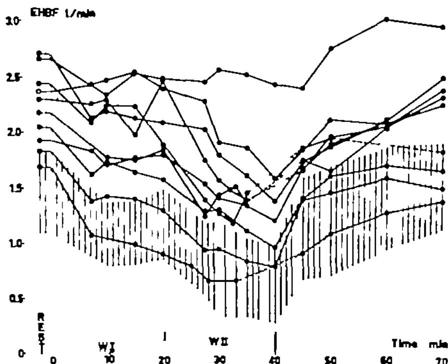


Fig 1 Estimated h patic blood flow (EHBF) et rest (pen ircles) duri g two supine 1 g bicycle work loads (WI and WII) and afte exercise (filled circle) i 10 patients Brok line indicate discontinued work load Hatched are represent the range f r 13 controls
(From P Lundbergh & T Strandell: Cha ges in hepatic circulation at rest during and fter exercise in young male with inf ction hepatiti compared with control Acta med scand In press)

comparing dye concentration in blood sampled from 15 controls at 45 and 50 minutes after the start of the infusion The coefficient of variation for a single determination thus including biological variation was 2 8 % f r arterial ICG concentration and 5 1 % for arterio-hepatic venous dye difference with no systematic change with time Generally a mean of 5 values from blood sampled between 30 and 50 minutes after the start of the infusion was used which reduced the error by a factor f 0 447

Hematocrit (%) was determined in duplicates on all arterial samples by spinning the blood f r 5 minute at 9000 g in a mi rohematocrit centrifuge (HB International Equipme t Co Needham Mass USA) The values were read under a magnifying glass The reproducibility was studied by comparing mean values f blood sampled in 24 subjects at 45 and 50 minute after the start of the ICG infusion The coefficient of variation f r a determination thus including biological variation wa 0 7 % with no systematic change with time

Plasma and blood volume (1) were estimated at rest by i v injection of about 3 μC ^{125}I human serum albumin. Afte an equilibration period of 10 minutes the activity of the indicator in blood was determined with a scintillator (Strandell & Lundbergh 1973) and the volumes were calculated with an assumed facto of 0 91 for total body hematocrit/large vessel hematocrit

Total hepati blood flow (l/min) at rest was calculated by the Bradley technique (Bradley et al. 1945) using, as recommended by Winkler t al (1965) the verage arterio-hepatic venous dye diff rence and the average change if any in arterial ICG conce tration The reproducibility wa studied by comparing values of blood sampled from 25 subject at 45 and 50 minut s after the start f the infusion The coefficient of variation f r a single d termination thus including biological variation wa 6 7 % with no systematic change with time As earlier mentioned individual mean values of generally 5 ICG det rminations were used, thereby reducing the error f the stimate by a factor of 0 447

During and f er xercise rre tions for variations in arterial dye concentrati due to change in plasma volume were performed mainly by

the method of Hultman & Castenf rs (1961) The reprodu ibility wa studied by comparing values f blood sampled from 22 subjects at 10 and 15 minute fte the start f the first work load The coefficient of variation for a single determination thu including bi logical variati wa 5 9 % with no systematic change with time The corresponding value at the second work load was 4 0 % (n=18) with significant change with time

Hepatic extra tion of ICG (%) was measured as the arterio-hepati venou differen divided by the arterial conce tration

Hemoglobin concentration (g/100 ml) and oxyge saturation (%) were calculated a mean values of 2-3 determination on a CO-oximeter (IL 182 Lexington Mass USA) calibrated against a standard f cyanmethemoglobin. The reproducibility of hemoglobin conce tratio s was studi d by comparing mean value f arterial and hepatic venous blood sampled from 23 subj cts t 45 and 50 minutes after the start of th ICG infusion The coeffici t f variation thus including bi logical variation wa 1 0 % with no systematic change with time The corresponding coeffici nt of variation for a determination of hepati venous oxygen saturation at re t was 1 8 % f r duplicate determination on the same blood it was 0 3 % Oxygen saturation value determined on arterial blood on the CO-oximeter (n=27) were not significantly diff rent (+ 0 7 %) from value btained on a Beckman-B spectrophotometer (Holmgren & Pernow 1959) Determination on hepati venous blood (n=27) with oxygen saturation value between 10 and 80 % were however significantly higher averaging 1 8 % The arterio-hepati veno s oxygen diff rence calculated from value derived on the CO-oximeter wa on an verage 4 % highe than when calculated from value d termined on the Beckman-B spectrophotometer; thi discrepancy wa not corrected f r

Splanchnic oxygen uptak (ml/min) was calculated a total hepati blood flow multiplied by arterio-hepatic venous oxygen difference

Total xygen uptake and carbon dioxide limination (l/min) were determined by the Douglas bag technique and micro-Sch lande analyse expired air being collected during 8-10 minutes at rest generally during 2-3 minute between the 16th and the 19th minute at the first load and during ½ - 1 minute between the 17th and the 19th minute of the second load

Cardiac output (l/min) was measured according to the direct Fick method by total oxygen uptake-determinations and calculations of arterio-venous oxygen difference from blood sampled from the pulmonary and brachial arteries

Heart rate (beats/min) was determined by electrocardiography

Intravascular pressures (mmHg) were measured by transducers (Bell & Howell no L 221 Woking Surrey England) using an EMMA-amplifier (SE labs Feltenham, Middlesex England) and a UV-writer (SE 3006 SE labs) Reference point for zero pressure was the midthoraci level of the sagittal chest diameter measured at the insertion of the fourth rib on the sternum Free and wedged hepatic venous pressures were repeatedly recorded at rest before exercise during the pause if any between the loads and for 30 minutes after exercise They were measured during quiet breathing and amplified so that 1 mmHg of pressure corresponded to a curve deflection of 1 cm. Individual mean value were calculated from which group means were derived The undamped and damped curve was analysed to check that the catheter was properly wedged For each measurement the pressure of the damped curve was integrated manually over 20-30 seconds and stated to the nearest 1/10 mmHg The reproducibility was studied by comparing the last two pressure recordings during the initial rest Th coefficient of variation for a single determination wa 6 0 % and 31 6 % (n=23) for wedged hepatic venous pressure and the difference between wedged and free hepatic venous pressure respectively There was no systematic difference between the first and the second recording for any of these two variables It was earlier (I) found that neither wedged h patic venous pressure nor the difference between wedged and free hepatic venous pressure changed significantly after exercise during the 30-minute period studied Therefore the individual mean values of all recorded pressures after exercise were used in further calculations

Splanchnic flow resistance (mmHg/l/min) was derived as the fall in pressure (i mean arterial blood pressure minu free hepatic venous pressure) divided by the total hepati blood flow

Postsinusoidal flow resistance (mmHg/l/min) was derived as the fall in

pressure (i wedged minus free hepati venou pressure) divided by the total hepati blood flow

Clearance f ^{85}Kr and ^{133}Xe Local blood flows can be studied by measurement f the tissue clearance of ^{85}Kr and of ^{133}Xe (Kety 1951 Lassen & Munck 1955), as the diffusion of the isotope i so rapid that at normal blood flows the exchange between tissue and blood is limited nly by the capillary blood flow and not by diffusi n barriers The solubility in blood of krypton and f xenon depends mainly on the actual hematocrit (Hardewig et al 1960 Kitani 1972) and the solubility in liver mainly on the hepati f t content (Andersen & Ladefoged 1967 Kitani & Winkler 1972) As krypton and xenon have markedly different solubility in fat difference in their partition coefficients between liver and blood have been used to calculate the hepatic triglyceride content (Kitani et al 1970)

^{85}Kr and ^{133}Xe (AB Atomenergi Studsvik Sweden) di solved in saline in glass syringe were used indicators A small portion f the live was labelled by slow retrograde hepatic venous injections f a 5-ml saline solution of the indicators with the hepatic venous catheter in the occluded position (Lassen 1965 Kitani t al 1970) Immediately after each inj ction which lasted f r 5-10 seconds the catheter was withdrawn under TV fluoroscopy to a free position and flushed with 20 ml of saline First one or two injections f about 1 mC of ^{85}Kr were given then two or three inj tions of a mixture f about 1 mC of ^{85}Kr and 0 02 mC f ^{133}Xe the time interval between two inj ctions wa 5-10 minutes

The hepati disappearance rate of the isotopes were simultaneously stimated from the count rate btained from 2 x 2 inche sodium iodide (T I) scintillator connected to a two-channel pulse-height analyser and counte (Picker Digital Dual Rate Computer) The energy windows f the pulse-h ight analyser were set to record the peak gamma radiation from the two isotopes The counts were accumul ted f periods f 6 seconds and printed on paper The first hepatic disappearance curve of ^{85}Kr alone were used to estimate the fraction f the krypton counts also recorded in the xenon window; thi fra tion wa used to obtain net xenon tivity After semilogarithmic pl tting the slope f the initial steepest exponential part f the disappearance curve wa calculated as $\ln 2/t_{\frac{1}{2}}$ where $t_{\frac{1}{2}}$ denotes the time in minute f r the activity to decrease to half The equations derived by

Kitani (1972) and Kitani & Winkler (1972) f r calculations of the solubility in blood and in liver of the isotopes and others used for calculation of the partition coefficient between liver and blood for krypton and for xenon as well as for clearance and estimated hepati fat content together with further details of the method, are given lsewhere (III)

The reproducibility was studied by comparing the half-times f r the isotopes d termined from the first and the second curve of simultaneously in ected krypton a d xenon The coefficient of variation f r a single determination of the half-time for krypton was 9 2 % and 7 2 % in patients a d controls respectively The corresponding values were 11 2 % and 9 5 % fo xenon and 4 7 % and 4 9 % for the quotient between the half-time for krypton and for xenon. The coefficient of variation for a single determination of th partition coefficient was 9 7 % and 7 8 % for krypton in patients and controls respectively as against 14 3 % and 12 7 % for xenon. Th corresponding values were 26 6 % and 24 9 % for stimated h patic fat content Individual mean values for half-times f the curves of krypton and xenon simultaneously injected were generally calculated which reduced these methodological errors by a fa tor of 0 707 or 0 577 There was no systematic difference between the first and the second determination for any of these variables

Biochemical liver tests Serum bilirubin serum glutami o transami ase (SGOT) serum glutamic pyruvic transami lactic d hydrogenase (LDH) alkaline phosphata 1 d termined at the La oratory of Clin by standard methods on blood sampled at diff rent intervals after exercise

HBAg testing Screening for HBAg and performed at t e Virus Department f t e tockholm County Council by a and immunoelectroosmophoresis (Berg

Statistical calculation were The following probability (P) levels $^{xxx}P < 0.001$ highly significant ^{xx}P probably significant Student s t-test

test were used as well as linear regression and correlation Multiple regression analyses were performed according to the method of least square

HEPATIC CIRCULATION AT REST

Previous investigations

In patients with viral hepatitis hepatic blood flow was first studied without hepatic venous catheterisation by intravenous inj ction of various substances extracted from the blood by the liver and determination of their hepatic learance Normal (Krook 1956 Neumayr 1956) or decreased (Leevy & Gliedman 1958 Burkle & Gliedman 1959) clearance was observed with colloidal radiogold (^{198}Au) and normal clearance (Schmitt 1964) with colloidal chromium phosphate (^{32}P) Y t the hepatic extraction f the substance used is not complete and thus these methods give only the clearance although the data were interpreted as hepatic blood flow The clearance is le s than the hepatic blood flow however since clearance = flow extraction

Normal values were calculated for hepatic blood flow both in the acute stage and after full convalescence after intrasplenic injections of radioactive iodinated serum albumin in 8 patients (Reichman et al 1957)

An adequate method for estimating total hepatic blood flow is by constant i v infusion f a dye such as bromsulphalein (Bradley et al 1945) r indocyanine green (Caesar t al 1961) hepatic venou catheterisation and repeated measurement f the arterio—hepatic venous extraction f the dye at a constant arterial level So far this method has been used in only one study in patients with viral hepatiti (Preisig et al 1966) yielding on an average normal hepati blood flow but greater than normal splanchnic oxygen uptake

Portal venous pre sure has been studied indirectly in patients with viral hepatitis to find out whether r not this pre sure would be affected by the disease By wedging a catheter in a hepatic vein the pressure in the sinusoids i transmitted by the stagnant column of blood and can thus be measured In the absence of presinusoidal block at which a normal wedged hepati venous pre sure can be found together with portal hyperte sion (Benhamou t al 1962) wedged hepatic venous pressure equals portal venou pressure This similarity has been stablished in man at various pressure levels both during operation (Paton et al 1953 Cohn et al 1954 Welch t al 1954) and in awak subjects (Storti t al

1966 Viallet et al 1970) Paton et al (1953) recorded normal value for wedged hepati venous pressure in 2 patients with acute hepatitis which indicated that the splenomegalia often attending hepatitis i not due to portal hypertension Subsequent studies have also shown normal (Myhre 1954 Preisig t al 1966) or slightly increased (Balfour t al 1954 Krook 1956 Leevy & Gliedman 1958) values f r wedged hepati venous pre sure in viral hepatitis

Portal venous pressure as judged from intrasplenic pressure measurements in 40 cases of acute hepatitis was slightly but consistently elevated in the acute stage of the disease; the average decrease at re-examinati n after full convalescence wa 5 cm H_2O (Reichman & Davi 1957) N sustained portal hyperte sion wa noted and none f the 21 patient studied by sophagoscopy had sophageal varice However sophageal varice have been bserved at esophagoscopy but not at X ray examination in 35 of 82 patients with acute viral hepatitis (Ha rter & Palmer 1959) Portal venous pressure a measured by needling of the varice in some of these patient was also moderately l vated but there are no further report on sophageal vari in acute viral hepatiti

A cordingly previous studi s in patient with acute viral hepatitis have shown signs of normal r decreased total hepatic blood flows and normal r slightly increased portal venous pressure

Present investigation

Table III summarize the data for the group of pati nts and controls (Papers II III) presented in Table IV-IX (Appendix) and in Table II (Paper III)

Estimated hepatic blood flow wa significantly higher in the pati nts than in the controls (Tables III IV) It wa not related to any of the body -size variables There wa no relation f any significance in the patients between hepati blood flow and the various biochemical liver tests,as a measure of the severity of the disease or the duration of jaundice

Tabl III Hepatic circulatory data and f t content in the group of patient and in the control group R = range and λ = partition coefficient between liver and blood Other symbols as in Tabl I

		Patient	Controls	Difference		P
Estimated hepatic blood flow l/min	$\bar{x}$	2 21	1.44	+0 77	+53%	xxx
	SD	0 34	0 21			
	R	1 69-2 71	1 08-1 82			
Oxygen saturation in arterial blood, %	$\bar{x}$	98 5	97 7	+0 8	+1%	>0 40
	SD	1 6	2 1			
	R	95 2-101 0	93 6-101 6			
Oxygen saturation in hepati venous blood %	$\bar{x}$	79 2	76 4	+2 8	+4%	>0 10
	SD	2.9	5 0			
	R	75 5-86 3	69 4-84 9			
Arterio-hepati venous oxygen difference ml/l	$\bar{x}$	36 1	43 0	-6 9	16%	x
	SD	4 8	7 8			
	R	27 6-44 9	32 0-56 2			
Splanchnic oxygen uptake ml/min	$\bar{x}$	79 7	60 9	+18 8	+31%	xx
	SD	16 0	9 2			
	R	60 9-109 4	48 8-74 4			
Hepatic extraction f 100 %	$\bar{x}$	54 2	75 3	-21 1	-28%	xxx
	SD	13 6	7			
	R	26 8-74 7	64 6-88 9			
Wedged hepati venous pressure mmHg	$\bar{x}$	10 1	8 7	+1 4	+16%	>0 10
	SD	2 2	1 7			
	R	8 3-14 2	5 6-11 8			
Diff rence between wedged and free hepati venous pressure mmHg	$\bar{x}$	2.9	0 9	+2 0	+220%	xxx
	SD	1 1	0 5			
	R	1 9-5 3	0 3-1 6			
Splanchni flow resistance mmHg/l/min	$\bar{x}$	37 5	60 0	22 5	-38%	xxx
	SD	8 9	9 7			
	R	29 1 54 1	48 7-82 9			
Po tsinusoidal flow resistance mmHg/l/min	$\bar{x}$	1 3	0 7	+0 6	+86%	xx
	SD	0 5	0 4			
	R	0 7-2 2	0 2-1 4			
λ Kr	$\bar{x}$	1.25	1 09	+0 16	+15%	xx
	SD	0 14	0 06			
	R	1 04-1 46	1 00-1 21			
λ Xe	$\bar{x}$	1 41	1 13	+0 28	+25%	xx
	SD	0 25	0 10			
	R	1 04 1.76	0 97-1 34			
Estimated hepatic fat content % wet live weight	$\bar{x}$	5 8	3 6	+2 2	+61%	xx
	SD	2 0	0 8			
	R	3 1-9 1	2.3-5 1			
Clearance Kr ml/g/min	$\bar{x}$	2 09	1.49	+0 60	+40%	x
	SD	0 66	0 41			
	R	1 51 3 41	0 89-2.04			

Oxygen saturation in arterial and in hepatic venou blood There was no ignificant diff rence between patients and control in oxygen saturation ither in arterial blood (Table III) o in hepatic venou blood (Tabl s III V)

Arterio-h patic venou oxygen difference was slightly lower in the patients than in the controls (Table III VI) The pati nts with the lowest value fo arterio-hepati venou xygen diff rence had the highest value fo serum bilirubin (r = -0 648^{x} n=10) and for SGPT (r= -0 671^{x}; n=10)

Splanchnic xygen utake wa significantly highe in the pati nts than in the controls (Tabl s III VII) and significantly correl ted to the biochemi al liver tests as measure f the severity f the disease Of the 10 patients those with the highest value fo splanchnic oxygen uptak had the highest value for serum bilirubin (r = 0 814^{xx}) fo SGOT (= 0 838^{xx}) and for SGPT (r = 0 853^{xx}); no relationship wa noted with the duration of j undice Splanchni oxygen uptake at rest as a fraction f total oxygen uptake was on an verage 0 34 (range 0 30 - 0 37) in 3 pati nts and 0 25 (range 0 20 - 0 29) in 4 controls

Hepatic extraction of ICG was d creased in all but 2 patient ; the average value was significantly lowe for the patients than fo the controls (Tables III VII) The patients with the highest value for serum bilirubin had the lowest hepatic extra tion of ICG (= -0 856^{xx}; n=10)

Wedged hepatic venou pre sure was similar in patients and controls (Tables III VIII) and there was no significant relationship between thi pressure and serum bilirubin SGOT SGPT r the duration f jaundice in any f the groups

Free hepati venous pre sure was also similar in both groups averaging 7 3 and 7 8 mmHg in patients and controls respe tively

Difference between wedged and free hepatic venou pressure was significantly highe in the patients than in the controls (Tables III VIII) The largest

pressure difference was noted in the 10 patients with the most abnormal values for serum bilirubin (r = 0 890^{xxx}) for SGOT (r = 0 883^{xxx}) and for SGPT (r = 0 666^{x}) There was no significant relationship between this pressure difference and total hepatic blood flow in the patients However the patients with the highest values for this pressure difference had also the highest values for splanchnic oxygen uptake (r = 0 761^{x}; n=10)

Splanchnic flow resistance was significantly lower in the patients than in the controls (Tabl e III IX) In the patients there was no significant relationship between splanchnic flow resistance and the various biochemical liver tests

Postsinusoidal flow resistance was significantly higher in the patients than in the controls (Table III). The patients with the highest values for postsinusoidal flow resistance had the highest values for serum bilirubin (r = 0 682^{x}; n=9) and for arterio-hepatic venous oxygen diff rence (r = 0 844^{xx}; n=9)

Clearance of ^{85}Kr and ^{133}Xe There wa no significant difference between the half-time of the two groups whereas the average quotient between the half-time for krypton and f r xenon wa significantly lower in the patients than in the controls (III) The partition coeffici nt between liver and blood f r both isotopes was significantly higher in the group f patients than in the controls as was the average estimated hepatic fat conte t 5 8 % as against 3 6 % (Table III) The hepati learance f krypton was also higher in the pati nts than in the controls (Table III)

As regards the relationship between estimated hepatic fat content on one hand and biochemical and physiological hepatic variables on the other the hepatic fat content wa highest in the patients with the least abnormal findings The pati nt with the highest hepatic fat conte t thus had the lowest values for serum bilirubin (r = -0 734^{x}; n=8) and f r SGOT (= -0 696^{x}; n=8) and the lowest values for the differe e between wedged and fre hepatic venous pressure (r = -0 775^{x}; n=8) and fo posts n soidal flow resistance (r = -0 682^{x}; n=7) No c rresponding relationships were observed in the group of patients between clearanc f krypton and the oth r variables

Re-examination of 3 patients 5 weeks after the first study showed that the values for all variable studied (IV) qualled the data in the earli r examined control group (II III)

Discu sion

The patients and the controls were studied by the same method The data in the controls were in good agreement with results obtained by others (Bradley 1949 Wade et al 1956 Bishop t al 1957 Bradley 1963 Rowell et al 1964 Wahren t al 1971 Kitani & Winkler 1972) and data at re-examination of 3 patients (IV) when they had almost completely recovered from the hepatitis were similar to results in the earlier studied control group (II III) Theref re the hepati circulatory and lipid abnormalitie observed in the patients during the acute stage of the disease seemed to be attributable to the disease r its treatment and not to selection However the di tary and medical treatment of the patients cannot have affected the hepati circulation significantly; only once or twice a week was the normal hospital di t replaced by a meal le rich in fat and the only medical treatment wa vitamin pills twice the daily requirement

The hyperkinetic hepati circulation wa not part of a general vasodilatation In 3 patients cardiac output at rest was on an average 8 7 l/min and hepatic blood flow 2 6 l/min The corresponding value f r 4 controls were 7 3 l/min and 1 5 l/min The average flow of blood to extrahepati parts f the body wa thus similar in both groups 6 1 l/min in the patient and 5 8 l/min in the controls

The high hepatic blood flows in the pati nts with hepatiti are in agreement with the hyperemi in the diseased organs in various infections In previous reports howeve total hepatic blood flow ha been stimated to be normal (Krook 1956 Neumayr 1956 Reichman t al 1957 Schmitt 1964 Preisig t al 1966) o decreased (Leevy & Gliedman 1958 Burkle & Gliedman 1959) Since all th se authors except Reichman et al (1957) and Preisig t al (1966) used the clearance method their values no not reflect hepatic blood flow Preisig t al (1966) in 14 patients with serum bilirubin between 2 4 and 32 0 mg/100 ml studied within an verage

of 19 days of the onset of hepatitis found total hepatic blood flow to be normal namely 1510 (890 - 2430) ml/min The discrepancy between their data and the present findings cannot be explained

It ha been shown in man that total hepati blood flow can increase markedly after typhoid vaccination even when fever was prevented (Bradley 1949) In rabbit an increased hepatic clearance of ICG wa observed for many weeks after i v injection of heterol gous protein (Lang t al 1965) which was considered to be due to an induced stimulation of the parenchymal cells maybe by a higher blood flow The present findings do not allow any conclusions as to whether or not immunologic factors contribute to the bserved high hepatic blood flows in acute viral hepatitis

A more probable cause of the high hepatic blood flow might be the unevenly di tributed hepatic cellular changes also impairing local irculation unevenly leading to anoxia in some areas A resultant metabolic vasodilatation might correspond to the most anoxic regions in the liver rather than to the average requirement of oxygen Thereby total hepati blood flow would be increased in excess of metaboli d mand resulting in a lower arterio-h pati venous oxyge difference and a higher oxygen saturation in the hepatic venous blood as in the present study Such a local h pati vasodilatation cannot be mediated by the portal vein since a decrease in the small portal venou flow resistance over the live is unlikely and anyhow cannot measurably in rease the portal ven us blood flow On the contrary an increased flow resistance over the portal sinusoids should be expected as in the sinusoidal type of portal hypertension (Rappaport t al 1970) and such an increased postsinusoidal flow resistance was also observed in the present tudy probably caused by swelling of the hepatocytes and/ changed mi rocirculation with an increased hepatic artery blood flow The changes seen at light microscopy of liver specimens from patient with viral hepatiti are similar irrespective of the type of th virus The observed change in hepatic circulation in the present patient with hepatitis A might also be expe ted in patients with other forms f hepatitis caused by viruses

In 1 patient the wedged hepatic venou pressure was recorded after exercise showi g marked wave resembling an arterial curve and a pressure

l vol close to the arterial one In some other patient unstable wedged pressure recordings were obtained after exercise showing sliding pre sures up to 30-40 mmHg which cannot represent portal venous pressure An altered hepatic microcirculation with increased hepati artery blood flow might explain these findings Although the absolute value of the difference between the patients and the controls wa low as regards the difference between wedged and free hepatic venous pressure it corresponded to twice as high a flow resistance in the patients a in the control Despite these significant hanges the wedged hepati venous pressure was not significantly higher in the patients than in the controls in agreement with the findings of thers (Paton et al 1953 Balfour et al 1954 Myhre 1954 Preisig et al 1966) Previously reported sign of increased portal venous pressure (Krook 1956 Reichman & Davi 1957 Leevy & Gliedman 1958 Haerte & Palmer 1959) probably indicate only somewhat more marked hepati changes than in the present study

The indication f higher than normal oxygen consumption during regeneration f hepatic ti sue (Brauer 1963) might explain the present increase in splanchnic oxygen uptake in agreement with the findings of Preisig et al (1966) In the present pati nts the values f the biocheni al liver test as measures f the degre f hepati bnormality were more cl sely correlated to the splanchnic oxygen uptake than to the total hepati blood flow probably owing to the fact that total hepatic blood flow was more influenced by ther factors g the sympathetic activity t the time f the tudy

The validity of the observed clearance of krypton a a measure of the average perfusion coeffi ient was tested by dividing the individual value of total hepatic blood flow stimated by the Bradl y technique (II) by the krypton learance The "liver weight thus calculated were significantly lower than the expected value f 1200 - 1600 g in normals (She lock 1968 Rappaport 1969) the average value being 1045 (range 700 - 1520) g in the controls and 1145 (range 690 - 1615) g in the patient The clearance f krypton thu overestimated the hepatic perfusion coeffici nt in the control by an average of 25 % po sibly because f local hyperemi in the injected area similar to what ha been bserved afte injection into skel tal muscl (Tönnesen & Sejrsen 1970) The previously reported range f hepatic clearance of krypton

(Kitani t al 1970) was of the same magnitude as that in the present control but lower than that in the patients

The amount of hepatic fat can be estimated in vivo in diff rent ways At microscopy of liver specimen no lipid is generally seen unless a high percentage of fat i present (Billing et al 1953) The fat content can be determined chemically after liver bi psy (Kramer et al 1969 Laurell & Lundquist 1971) but in the individual case the representativity of this small specimen may be questioned During a hepatic blood flow study and without liver biopsy (III) it is pos ible to stimate the hepati triglyceride content (Kitani & Winkler 1972) This seems to be appropriate although other lipids such as cholesterol and phospholipid with diff rent solubility for the isotopes are also present in the liver since the greatest variation in hepatic fat content in healthy subj cts and spe ially in patients with liver disorders is caused by triglyceride (Klenk 1964 Kramer et al 1969)

An increased hepatic fat ontent has been observed in viral hepatitis both in the acute stage (Christoff rsen 1973) and during restitution (Colwell 1954) In the present series the hepatic fat content was highest in the pati nt with the l ss marked abnormalities f the biochemical liver tests and of the circulatory data (III) probably because these pati nt were studied in a later stage of the disease The hepatic fat content wa normal at re-examination of the 2 subjects (IV) who had had the highest values (III) This temporary increase in hepati fat content might depend on di tary factors (Colwell 1954 Laurell & Lundquist 1971) Another explanation could be a temporary metabolic insufficiency f the regenerating hepatocyt s

As no major change in central circulation were observed in the acut stage f hepatitis A (II) it seems very unlikely indeed that a short period f bed rest ad libitum could in itself ause increases of hepatic blood flow splanchnic oxygen uptake and hepati fat content Thus the changes observed in the present cases during hepatitis A should be related to the disease Judged from these few pati nt in the present study the hepatic ircul tory and lipid abnormalitie during acute stage f uncomplicated hepatitis might be expected to disappear within 2 months f the onset of symptoms This i in good agreement with the finding that the histo-l gical change in viral hepatiti are usually restituted 2-3 months after onset of the disease (Wepl r & Wildhirt 1968)

HEPATIC CIRCULATION DURING AND AFTER EXERCISE

Previou investigation

When healthy subj cts perform physical exercise total hepatic blood flow i reduced (Bradley 1949) th heavier the exercise the greater the flow reduction (Wade et al 1956 Bishop et al 1957) During maximal work in the upright position total hepati blood flow has been found to be only 20 % of the value at rest (Rowell t al 1964) This decrease in hepatic blood flow during exercise has been found to be more closely related to the relative than to the bsolute work load of the individual (Rowell et al 1964)

In patients with viral hepatitis the effect of exercise on hepati blood flow has not been tudied directly So far the only ffects that have been studi d are those on recovery and relapse rate Swift t al (1950) and Krikler & Zilberg (1966) report negative effects on the liver of exercise perf rmed in the acute stage of the disease Repsher & Freebern (1969) and Edlund (1971) on the other hand found no undue ff cts on the liver after strenuous physical exercise Swift t al (1950) noticed that the mean duration of the disease was about 2 weeks longe afte exercise in patient with serum bilirubin value above 3 mg/100 ml than in those with values lower than 3 mg/100 ml Krikler & Zilberg (1966) noted deleterious ffe ts of physical exercise in that 3 of their 5 patients who performed strenuou exercise in the pre-icteric stage of the hepatitis died within 4-16 days f the onset f the disease but give no data on the numbers of patient with hepatitis who perform physical exercise during the pre-i teric period f the disease without negative eff ts on recovery and relapse rate Repsher & Freebern (1969) investigated 398 American servicemen with hepatiti A in Vietnam; all f them had increased level f serum bilirubin and SGOT onset of symptoms l ss than 14 days before admission and an asymptomtic period f less than 5 days One-half of the me were ambulant in the ward without prescribed exercise The other half had daily exercise fo 3 hours a noon their symptoms were considered to be slight irrespective f the degree of the abnormaliti s in their biochemical liver test ; average serum bilirubin was 7 5 mg/100 ml No adverse effects of the exer-ise were noted on recovery r relapse rate However neither the material no the intensity of the exercise was clearly defined Edlund (1971) found

no d layed recovery and no deleterious ffe ts on the liver in 23 patients with acute viral hepatitis who when SGPT was below 300 unit performed a moderately heavy bicycle exercise for 12 minutes on 6 consecutive days

Present inve tigation

The 10 patient and 13 controls were studied during two 20-minute periods of supine l g exercise and fo 30 minutes thereafter (I II) The average heart rate was 126 beats/min and 116 beats/min in patients and controls respectively at the end f the first work load averaging 380 and 496 kpm/min (Table II X) At the end of the second work load the average heart rate was 166 beats/min and 175 beats/min for patients and controls respectively at 720 and 985 kpm/min (Tables II X) The difference in heart rate betwee the groups was not significant at any of the loads The first 4 patie t studied had heart rates not exceeding 160 beat /min as had also 1 co trol In relation to work load the mean heart rate f the patients was thu about 15 beats/min higher than that of the ontrol (Fig 2)

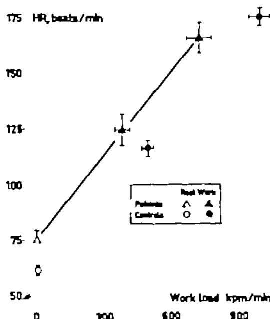


Fig. 2 Heart rate (HR) at re t (por symbols) and at the end f two generally 20-minute period of supine leg exercise (filled symbols) in relati to work load in 10 patients (triangl s and solid line) and in 13 controls (circle and broken lines) Gro p mear and standard rror f the mean are given

Hepatic blood flow During the first work load total hepatic blood flow decreased by an average of 15-20 % in both groups (Fig 1 Table IV); the diff rence in absolute flow value between the groups was highly significant Even lower total hepati blood flows were observed in all patients but one no 3 during the second load veraging 1 30 l/min and 0 56 l/min respectively in patients and controls (Table IV) or 59 % and 39 % of the respective value at rest The h patic blood flow for both groups is shown in Fig 3 in relation to absolute work load and in Fig 4 to heart rate a a measure f the relative work load When the reduced hepatic blood flow during exercise was expressed in per cent of the value at rest th patient and the controls had the same flow redu tion in relati n to the increase in heart rate (Fig) a a measure of the relative work load The increase in splanchni flow resistance with exercise was not significantly diff rent between patients and controls when related to the simultaneous increase in heart rate

At 30 minutes afte exercise the value f r total hepati blood flow (Table IV) mean arterial blood pre sure (Table II) and splanchnic flow resistance (Table IX) had returned to the initial value at rest in both group but the heart rates (Table X) were still significantly el vated above initial resting value

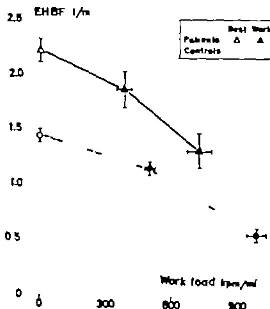


Fig 3 Estimated hepatic blood flow (EHBF) t rest and t the end of two generally 20-minute periods f supine leg exercise in relation t work load in 10 patients and 13 controls Group mean and tandard rrors f the mean are given Symbol as in Fig 2

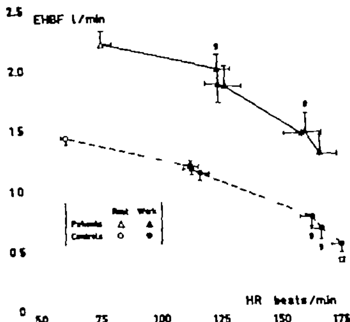


Fig 4 Estimated hepatic blood flow (EHBF) at rest after 10 and 15 minute and at the end of two generally 20-minute periods of supine leg exercise in relation to heart rate (HR) in 10 patients and 13 controls Group means and standard errors of the mean are given No of individuals is set out when reduced Symbols as in Fig 2 (Reproduced from Paper II)

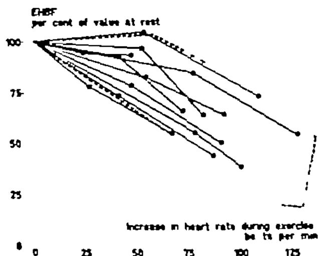


Fig. 5 Change in estimated hepatic blood flow (EHBF) during xercise (end of two generally 20-minute periods of supine leg exercise) in relation to change in heart rate in 10 patients Area within broken line represents range f r 13 controls (Reproduced from Paper II)

Hepatic extraction In the patients the average hepatic venous oxygen saturation wa reduced from 79 % at rest to 74 % and 60 % at the end of the first and second work loads respectively The corresponding value for the controls were 76 % at rest and 70 % and 36 % during exercise (Table V) Oxygen saturation in arterial blood did not differ between the groups at any f the loads and was not significantly diff rent from the values at rest The resultant arterio-hepatic venous oxygen difference wa lower in the patients than in the controls during both loads (Fig 6 Table VI) Splanchnic oxygen uptake did not change significantly in any of the groups during exercise compared with that t rest (Table VII) Accordingly the increase in arterio-hepatic venous oxygen difference during exercise balanced the decrease in total hepatic blood flow

At 30 minutes after exercise hepati venou oxygen saturation (Table V) and arterio-hepati venous oxygen diff rence (Table VI) were still significantly changed compared with the values at rest in the group f patients but not in the group f controls

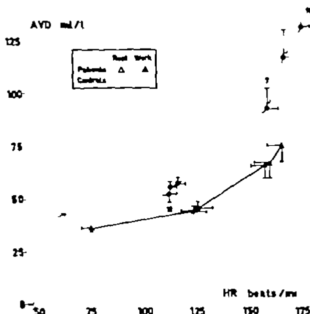


Fig. 6 Arterio-hepati venous oxygen diff rence (AVD) at rest after 10 and 15 minutes and at the end f two generally 20-minute periods of supine leg xercise in relation to heart rate (HR) in 10 patients and 12 controls Group means and standard rrors f the mean are given No f individual is set out when reduced Symbols as in Fig 2 (Reproduced from Paper II)

Hepati venous pre sures In both groups wedged and especially free hepatic venous pressures were lower when measured in the 30-minute period after exercise (Table VIII) During the 15-minute pause between the loads the average wedged hepati venous pressure wa unchanged compared with the value before exercise in patients and controls thus studied In both groups the average diff rence between wedged and free hepatic venou pressure in reased after exercise but this change was significant for the ontrols alone After the first load the average pressure difference was virtually unchanged compared with that at rest 3 4 mmHg and 2 9 mmHg respectively in the patient and 1 1 mmHg and 0 9 mmHg in the controls

Effect of exercise on symptoms and biochemical liver test In the 8 patients who at the time of the study had serum bilirubin values below 4 mg/100 ml and SGOT and SGPT below 150 units and 400 unit respectively no negative ffects of the exercise were noted on recovery or relapse rate (II) Of the other 2 patients no 3 showed a normal recovery and the values of his biochemical liver tests decreased except for a slight and temporary increase of SGOT from 300 to 390 units between the 4th and the 6th day Thereafter SGOT decreased normally and the recovery was uneventful Thi patient wa the only one in whom no decrease of hepatic blood flow was noted during exercise and in whom a flow increase above the resting value was recorded after exercise In patient no 6 the symptoms subsided and on the 4th day serum bilirubin was 8 4 mg/100 ml wherea SGOT and SGPT both were 1000 units On the 6th day the corresponding values were 7 1 960 and 1000 Te days after the catheterisation the patient experienced malaise fatigue lo s of appetite and increased jaundice By the 11th day serum bilirubin had risen to 11 3 mg/100 ml whereas SGOT and SGPT had decreased to 700 units and 840 unit respectively One week later he began to improve clinically in spite of a serum bilirubin value of 13 1 mg/100 ml; SGOT and SGPT were down to 460 units and 490 units respectively Thereafter serum bilirubin decreased and the recovery wa uneventful and the patient was discharged 5 weeks afte theterization

Discussion

The patients and the controls were studied at similar heart rates the values for the patients being somewhat lower than those f r the controls mostly owing to the patient lower working apacity (Fig 2) but also to the f ct that the first 4 pati nts were studied at a capacity lower than maximal Since the pertinent comparison during exercise between patient and controls were made in relation to heart rate as a measure of relative work load (Sjöstrand 1960) the present diff rences in work load between pati nts and controls are of minor importance The average difference in heart rate in relation to work load f about 20 beats/min between the acute stage and convalescence in th 3 pati nt who were re-examined (IV) was similar to the diff rence between the 10 pati nts and the 13 controls (II) Therefore the higher heart rate during exercise f the pati nts compared with those f the controls were probably caused by the disease r its treatment by bed-rest rather than by selection r by diff rence in premorbid physical capacity

In spite of great diff rences in flow value between pati nts and controls and signs of in reased hepatic artery blood flow in the patients the decrease in total hepatic blood flow during exercise and the increase in splanchnic flow resistance were equal in both groups in relation to the increase in heart rate indicating the same sympathetic flow control in both groups (Fig 5) There were no signs f the splanchni oxygen uptak being affected in the patients by the decrease in hepati blood flow during exercise

It has been suggested that the increase after exercise in the diff rence between wedged and free hepatic venous pressure might be related to the increased postsinusoidal flow re istance due to swelling of the hepatocyte possibly voked by ischemia during the exercise (I) In the present patients with hepatitis no such increase in pressure difference wa noted after xercise The 2 patients nos 3 and 6 who had the highest value of serum bilirubin at the time f the study had also the highest difference between wedged and free hepatic venous pressure but only an average increase of thi pressure difference afte exercise In one of these patients no 3 an increase f hepatic blood flow above the resting value wa noted after exercise which might be interpreted as a sign of post-exercise metabolic vasodilatation Thus the present study does not f vour the view that the

increase in postsinusoidal flow resistance after exercise is related to hepatic ischemia but seems to show that the increase is related to altered hepatic microcirculation due to other causes

None of the present 8 patients with values for serum bilirubin below 4 mg/100 ml and for SGOT and SGPT below 150 units and 400 units respectively at the time of the catheterization showed any symptoms or signs of negative effects of the exercise Of the 2 patients nos 3 and 6, with serum bilirubin values of 5 8 mg/100 ml and 9 6 mg/100 ml respectively no 3 showed a slight delay in the decrease of SGOT and no 6 a relapse that delayed his recovery for about 2 weeks Patient no 3 performed only 500 kpm/min with a final heart rate of 120 beats/min whereas patient no 6 wa well-trained and performed a heaviest load of 1000 kpm/min with a final heart rate of 180 beats/min This prolonged recovery may have been due to chance since according to current textbooks (Sherlock 1968 Mosley & Galambos 1969) and the experi nce in this hospital this clinical course occurs in about 10 % of patients with acute viral hepatitis However if they are not due to chance these increases in values for biochemical live tests after exercise in 2 patients show that signs of liver cell damage can be provoked during exercise even if total hepatic blood flow is kept within normal resting limits This might be explained by heterogeneity of hepatic blood flow and local areas of ischemia Thus the po sibility that the physical exercise in these 2 patient may have been the provoking factor cannot be excluded and pending more extensive studi s heavy physical exercise in hepatitis A should be avoided until g serum bilirubin is le s than about 4 mg/100 ml This supports Swift s et al (1950) conclusion that in patients with serum bilirubin values lower than 3 mg/100 ml exercise did not prolong the duration of the disease

GENERAL SUMMARY

The tudy comprised 10 male patients and 17 healthy male volunteers as controls All were between 20 and 30 years old with similar degree of physical training; none had a history of drug or alcohol abuse and all were hepatitis-B-antigen-(HBAg)-negative The patients were treated f r acute ictori viral hepatiti at the Roslagstull Hospital Stockholm, over the period April 1972 - October 1973 Although being sporadic the cases were diagnosed as hepatitis A since clinical and laboratory findings were not consistent with infection due to Epstein-Barr virus or cytomegalovirus the onset of disease was acute and the pati nts were HBAg-negative as early as 5 (2-8) days after onset f jaundice

Hepatic circulation was studied in the supine position by hepatic venous catheterization at rest and during two generally 20-minute periods f supine l g exercise and f r 30 minute thereafter The second work load wa twice s high as the first one and chosen so as to correspond to the heavi st load the subject was expe ted to sustain f r another 20 minute Total hepati blood flow was stimated according to the Bradley technique with i v constant infusion of indocyanine green dye Arterio-hepati venous oxygen diff rence was determined and splanchnic oxygen uptake calculated Wedged and free hepati venous pressures and the diff rence between them were measured and splanchnic flow resistance and post-sinusoidal flow resistance calculated In 8 patients and 10 control inj ctions of a solution of ^{85}Kr and ^{133}Xe in saline were also given at rest into an o cluded hepati vein and the disappearance rates of the isotope were measured with an external scintillator over the liver and given s the half-times of the initial steepest exponential part of the disappearance curve The triglyceride part of the hepatic fat content was estimated from actual hematocrit and the quotient between the half-time for krypton and for xenon. Partition coefficients between liver and blood were calculated as well as clearance of krypton

At rest in the supine position the patients had significantly higher values than the controls fo total hepatic blood flow (2 21 l/min as against 1 44 l/min) for splanchnic oxygen uptake (79 7 ml/min versus 60 9 ml/min) fo the diff rence between wedged and free hepatic venous pressure (2 9 mmHg as against 0 9 mmHg) and for estimated hepatic fat content (5 8 % versus 3 6 %) Arterio-hepati venous oxygen difference splanchnic flow resistance

and hepatic extraction of ICG were lower whereas the clearance of krypton was higher in the group of patients than in the control group

At re-examination of 3 patients 5 weeks after the first study all values had decreased markedly as compared with the first study hepatic blood flow and splanchnic oxygen uptake by 20-25 % and all values qualled those of the control group

During supine leg exercise the 10 patients and 13 controls were studied at final heart rates averaging 166 beats/min and 175 beats/min respectively at the heavi st work load of 720 kpm/min and 985 kpm/min The difference in heart rate was not significant but in relation to work load the pati nts had about 15 beats/min higher heart rates than the controls During exercise total hepatic blood flow decreased in both groups by about 0 8 l/min When the reduced hepatic blood flow during exercise was expressed in per cent of the value at rest the patients and the controls had the same flow reduction and increase in splanchnic flow resistance in relation to the increase in heart rate as a measure of the relative work load Arterio-hepatic venous oxygen difference during exercise was lower in pati nts than in controls The increase with exercise balanced the decrease in hepatic blood flow indicating an unchanged splanchnic oxygen uptake during exercise

After exercise wedged hepatic venous pressure was lower in both groups but since free hepatic venous pressure was still lower the diff rence between wedged and free hepatic venous pressure was higher in both groups signifi antly so in the controls At 30 minutes after exercise the values fo total hepatic blood flow and splanchnic flow resistance had returned to the initial values at rest in both groups Arterio-hepatic venous oxygen difference and heart rates were however still significantly higher than the initial resting values in the group of patients

No negative ffects of the exercise were noted in the 8 patient with values f r serum bilirubin below 4 mg/100 ml whereas a temporary increase r delayed decrease of both serum transaminases and serum bilirubin were noted in the remaining 2 patients with serum bilirubin values of 9 6 mg/100 ml and 5 8 mg/100 ml respectively

A probable xplanation of the high hepatic blood flow seen in the acute stage of h patitis A in the present study might be that because of the

uneven distribution of the hepati cellular changes local irculation would be unevenly impaired and anoxia thu develop in some area with a resultant metabolic vasodilatation The increase in regeneration of liver cells might have contributed as both total hepatic blood flow and splanchnic oxygen uptake were elevated There were no signs indicating that the splanchnic oxygen uptake wa affe ted by the decrease of hepati blood flow during exercise The increase of hepati fat content might depend on di tary factor and/or a temporary metabolic insufficiency f the regenerating hepatocytes

The fact that the decrease in hepati blood flow and the in rease in splanchnic flow resistance with exercise were similar in patients and controls indicated the same sympathetic flow control during exercise in both groups

It was concluded that the observed change in the acute stage f hepatiti were attributable to the disease and that they were reversible Pending more extensive studies strenuous physical exercise should be avoided in patients with hepatiti A in the acute stage g until serum bilirubin is below 4 mg/100 ml

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APPENDIX

Tabl IV Estimated hepatic blood flow (l/min) at rest (RI) t 10 and 15 minute and t the end of two generally 20-minute periods of supine leg xercise (WI and WII) and at 5 10 20 and 30 minutes afte the exercise (RII) in 10 pati ts and 13 control Data at rest are given f r 4 controls in whom values during exercise had to be discarded $\bar{X}$ = group mean value SD = standard devi tion n = no of individuals xxx = P <0 001 xx = P <0 01 and x = P <0 05 where P i the probability that the diff rence between patients and controls wa caused by random f tors Figure within parenthese are not i cluded in mean values

	RI	WI			WII			RII			
		10	15	20	10	15	Final	5	10	20	30
Pati nt no 1	1 92	1 70	1 77	1 79	1 40	1 33	1 40 [a])	1 66	1 89	2 08	
2	2 29	2 29	1 97	2 46	1 56	1 48	1 37)	1 65	1 94	2 04	2 37
3	2 65	2 33	2 52	2.48	2 6	2 51	2 43	2 39	2 74	3 06	2 94
4	2 71	2 19	2 12	2 09	1 79	1 60	1 37 [a])	1 73	1 86	2 08	2 47
5	2 18	1 77	1 74	1 84	1 42	1 50	1 36 [a]}	1 74	1 64	2 03	2 31
6	2 44	2 24	2 23	1 88	1 26	1 17	1 45 [a])	1 85	1 94	-	1 83
7	2 36	2 47	2 53	2 39	1 90	1 85	1 58	1 83	2 10	2 06	2 24
8	2 05	1 72	1 63	1 57	1 29	1 10	0 95	1 38	1 60	1 69	1 64
9	1 83	1 42	1 39	1 29	0 93	0 82	0 78 [a])	1 38	1 43	1 57	1 48
10	1 69	-	0 98	0 89	0 65	-	0 54)	0 89	1 07	1 22	1 39
$\bar{X}$	2 21	2 01	1 89	1 87	1 48	1 48	1 30	1 65	1 82	1 98	2 07
SD	0 34	0 37	0 49	0 52	0 53	0 49	0 51	0 39	0 44	0 50	0 52
n	10	9	10	10	10	9	10	10	10	9	9
Control no 1	1 43	1 23	1 25	1 20	0 89	0 80	0 63 [a])	1 40	1 54	1 63	1 63
2	1 49	1 11	1 10	0 89	0 45	0 31	0 28 [a]}	0 69	0 69	0 95	1 10
3	1 46	1 09	1 09	1 08	(0 20)	-	0 50 [a])	0 82	0 97	1 21	1 43
4	1 31	1 22	1 38	1 32	1 06	1 03	0 80	1 30	1 47	1 39	1 40
5	1 08	0 78	0 79	0 84	0 41	0 33	0 26 [a])	0 80	0 81	0 92	
6	1 10	1 30	1 02	0 80	(0 60)	-	0 60 [a]}	0 66	0 98	1 09	1 28
7	1 82	1 30	1 24	1 12	0 76	0 71	0 67 [a])	1 05	1 36	1 52	1 64
8	1 43	1 35	1 22	1 25	0 85	0 72	0 63 [a])	1 23	1 24	1 20	1 38
9	1 51	1 30	1 10	1 02	0 68	0 61	0 53)	1 33	1 40	1 25	1 56
10	1 63	1 35	1 37	1 48	1 18	1 01	0 97	1 58	1 68	1 86	1 89
11	1 33	1 18	1 21	1 28	0 78	0 67	0 52)	1 22	1 30	1 21	1 46
12	1 66	1 20	1 35	1 27	(0 30)		0 30 }	0 96	1 08	1 40	1 73
13	1 46	1 24	1 27	1 41	(0 62)			1 03	1 19	1 28	1 21
14	(1 60)										
15	(1 38)										
16	(1 68)										
17	(1 09)										
$\bar{X}$	1 44	1 20	1 18	1 15	0 78	0 69	0 56	1 08	1 21	1 30	1 48
SD	0 21	0 15	0 16	0 22	0 25	0 2*	0 21	0 29	0 29	0 26	0 23
n	13	13	13	13	9	9	12	13	13	13	12
Diff rence	0 77	0 81	0 71	0 72	0 70	0 79	0 74	0 57	0 61	0 68	0 59
P	xxx	xxx	xxx	xxx	xx	xxx	xxx	xxx	xxx	xxx	xx

[a])Exercise terminated afte 10-18 minutes because of f tigue

Table V Oxygen saturation in hepatic venous blood (%) at rest (RI) at 10 and 15 minutes and at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and at 5 10 20 and 30 minutes after the exercise (RII) in the patient and the controls Data at rest are given for 4 controls in whom values during exercise had to be discarded Figures within parentheses are not included in mean values Other symbols in Table IV

	RI	WI			WII			RII			
		10	15	20	10	15	Final	5	10	20	30
Patient no 1	78 1	75 9	77 1	75 9	71.4	72 2	67 3)	76 5	75 9	78 7	
2	79 5	76 8	77 9	74 4	69 0	66 4	66 7)	73 2	71 6	71.9	73 4
3	77 0	76 1	75 8	75 0	75 6	75 7	6 4	76 5	76 1	73 5	73 6
4	86 3	84 8	84 2	84 0	80 4	77 1	75 0)	80 6	80 9	82 0	83 4
5	80 1	74 9	72 3	73 1	55 4	54 2	49 9 a)	75	75 4	77 6	79 5
6	77 9	74 3	76 0	74 6	60 6	56 8	53 9 a)	71.2	69 7		69 0
7	80 4	81 2	81 2	80 8	74 6	73 9	69 1	74 9	75 6	73 2	76 6
8	78 4	68 7	69 8	68 6	58 0	54.3	52 6	65 3	64 5	67 0	68 3
9	78 6	71 0	72 6	68 1	50 5	49 2	44.7 a)	64 4	64 5	68 1	69 7
10	75 5	-	73 2	68 6	49 4	-	41 5 a)	64 2	68 5	71 3	73 2
$\bar{x}$	79 2	76 0	76 0	74 3	64 5	64 4	59 7	72 2	72 3	73 5	74.1
SD	2 9	4 8	4 4	5	11 1	10 8	12 7	5 8	5.4	4.7	5 0
n	10	9	10	10	10	9	10	10	10	9	9
Control no 1	73 0	-	70 4	67 3		43 5	35 9 a)	65 7	68 0	70 8	70 0
2	75 1		66 5	67 2	-	8 2	7 6 a)	35 1	48 4	48 0	59 5
3	(77 9)	(68 6)	(64 1)	(62 4)	-	-	-	-	-	-	-
4	69 4	69 3	70 6	65 0	60 0	40 9	45 4	70 3	73 3	70 9	74 6
5	69 8	59 0	58 7	58 4	30 5	19 9	11 9)	48 3	52 3	61.8	70 3
6	75.4	80 5	79 9	79 1	(48 6)		48 6)	74 6	71 7	75 2	79 9
7	84 9	82 6	78 8	78 0	58 0	53 3	48 6	70 6	73 6	74 3	75 5
8	82 1	78 8	77 1	78 5	62 3	55 7	44 0)	73 2	72 8	76 9	77 5
9	82 6	75 1	73.4	74 6	51 3	49 6	44.1)	72 9	74 1	77 0	81 0
10	78 7	74 0	74 4	73 9	71.3	65 1	67 5	71 3	75 2	76 0	79 9
11	72 2	66 7	65 7	64 4	46 3	37 0	23 2 a)	59 6	59 0	62 6	65 8
12	78 2	72 6	69 5	68 4	(18 0)		18 0 a)	50 5	74 9	80 2	81 8
13	74 8	71 5	69 9	68 5	(44.1)	-		63 7	67 1	70 1	70 2
14	(67 3)										
15	(72 7)										
16	(82 1)										
17	(70 8)										
$\bar{x}$	76 4	73 0	71 2	70 3	54 2	42 6	35 9	63 0	67 5	70 3	73 8
SD	5 0	7 0	6 0	6 5	13 2	18 2	18 5	12 4	9 3	9 0	6 9
n	12	10	12	12	7	9	11	12	12	12	12
Difference	2 8	3 0	4 8	4 0	10 3	21 8	23 8	9 2	4 8	3 2	0 3
P	>0 10	0 30	x	0 10	>0 10	xx	xx	x	>0 10	>0 30	0 90

a) Exercise terminated afte 10–18 min te because of fatigue

Table VI Arterio-hepati venou oxygen diff rence (ml/1) at rest (RI) t 10 and 15 minutes and at the nd f two generally 20-minute periods f supin 1 g exercise (WI and WII) and at 5 10 20 and 30 minute after the xercise (RII) in the pati t and the controls Data at rest are given for 4 control in whom values during exercise had to be discarded Figure within parentheses are rot included in mean value Other symbols a in Tabl IV

	RI	WI			WII			RII			
		10	15	20	10	15	Final		10	20	30
Pati nt no 1	31 6	37 2	35 6	37 8	47 0	44 7	51 6 a)	31 9	32 1	29 2	-
2	35 8	42 5	40 9	45 *	57 8	62 6	60 9 a)	48 4	51 5	48 7	44 0
3	39 3	43 8	44 2	43 9	4 9	44 4	43 0	43 1	42 2	46 2	44 2
4	27 6	31 4	33 6	33 1	41 3	48 9	3 7 a)	39 9	38 0	33 9	31 2
5	37 5	49 6	55 7	49 0	88 3	93 6	102 8 a)	48 0	45 2	39 7	35 8
6	44 9	54 0	51 0	51 1	82 8	88 6	94 9 a)	56 4	56 5		54 1
7	31 9	32 0	31 6	31 9	42 1	43 7	50 7	40 7	38 2	41 3	36 0
8	37 8	56 3	53 5	55 5	75 5	82 8	83 8	58 5	60 0	54 6	51 9
9	30 2	50 4	47 2	55 2	89 0	91 8	101 2 a)	63 1	60 6	52 9	49 8
10	38 2	-	45 2	53 2	93 2	-	106 8 a)	60 2	51 1	44 7	39 3
$\bar{X}$	35 1	44 1	43 9	45 7	66 0	66 8	74 9	49 0	47 5	43 5	42 9
SD	4 8	9 2	8 4	8 8	21 8	22 2	25 3	10 3	9 9	8 4	8 0
n	10	9	10	10	10	9	10	10	10	9	9
Control no 1	48 7	-	57 2	60 5	-	115 2	129 3 a)	62 4	59 0	48 9	52 1
2	46 3	-	62 1	60 3	-	194 3	191 3 a)	131 1	91 2	90 1	63 2
3	(44 0)	(64 3)	(64 8)	(70 6)	-		-		-	-	
4	56 2	57 1	55 6	67 1	78 6	97 4	109 0	57 6	50	52 5	46 9
5	51 4	80 3	80 7	82 2	142 7	167 7	181 3 a)	102 0	92 1	71 0	54 9
6	50 1	43 0	43 7	46 0	(123 6)		123 6 a)	59 6	61 0	50 2	42 2
7	32 0	38 2	46 2	46 7	86 0	96 7	105 2 a)	59 2	52 8	51 0	49 1
8	34 0	41 0	44 4	40 3	73 4	87 2	109 4 a)	48 9	50 1	40 9	38 5
9	33 8	0 8	54 5	52 2	104 1	104 8	117 2 a)	54 6	51 3	44 2	36 2
10	45 7	8 5	57 8	58 3	68 2	81 3	74 9	63 6	56 8	54 9	45 1
11	37 9	52 9	54 6	57 2	94 0	114 1	138 9 a)	65 4	65 6	57 0	50 9
12	39 3	51 4	56 3	59 0	(159 8)		159 8 a)	90 8	45 1	34 0	31 2
13	41 1	47 8	0 7	53 3	(99 2)	-	a)	62 1	54 7	48 0	47 0
14	(42.2)										
15	(56 7)										
16	(30 2)										
17	(63 0)										
$\bar{X}$	43 0	52 1	55 3	56 9	92 4	117 6	130 9	71 4	60 9	53 6	46 4
SD	7 8	11 9	9 8	10 9	25 3	38 2	34 7	24 1	15 4	14 6	8 7
	12	10	12	12	7	9	11	12	12	12	12
Difference	-6 9	-8 0	11 4	-11 2	-26 4	0.8	-56 0	-22 4	-13 4	10 1	3 5
P	x	>0 10	xx	x	x	xx	xxx	x	x	>0 05	>0 30

a) Exercise terminated fte 10-18 minute because f fatigue

Table VII Splanchni oxygen uptake and hepati extraction f ICG at rest (RI) t the end of two generally 20-minute period of supir leg exercise (WI ard WII) and t 30 minutes fter the exercise (RII) in the patients and the controls. Data at rest are given for 4 controls in whom value during exercise had to be discarded. Figures within parentheses are not included in mean value Other symbols as in Table IV

		Splanchnic oxyge uptake ml/min				Hepati extraction f ICG %			
		RI	WI	WII	RII	RI	WI	WII	RII
Patient no	1	60 9	67 6	61.6	-	59 2	54 1	53 1	-
	2	82 9	112 6	83 3[a]	104.1	57.1	54 1	60 1[a]	50 0
	3	104.2	108 7	106 3	130 1	37 7	34 0	30 7	26 2
	4	74.6	69 1	73 4	77 1	74.7	73 2	74.3	69 4
	5	81.7	90 1	140 2[a]	82.4	64.1	60 2	56 8[a]	49 0
	6	109.4	95 3	137 0	98 8	26 8	23 8	25 0[a]	22 3
	7	75 2	76 4	80 0	80 4	58 8	47 8	47 4	51 9
	8	77 3	87 0	79 7	85 3	58 4	59 8	66 0	55.4
	9	66 6	71 1	78 6	73 7	57 9	60 8	69 8	54 2
	10	64 5	47 3	57 6	54 7	47 4	56 3	62 6	44.5
	X̄	79 7	82 6	89 8	87 4	54 2	52 4	54 6	47 1
	SD	16 0	20 2	28 9	21.4	13 6	14 2	16 2	14 7
	n	10	10	10	9	10	10	10	9
Control no	1	69 8	72 5	80 9	85 0	72 8	61 7	58 5	52.1
	2	68 8	53 5	54.0[a]	69 8	65 6	68 3	68 6	60 9
	3	(64.4)	(75 9)	-	-	64 6	66 2	76 4	55 4
	4	73 7	88 5	87 5	65 6	80 9	58 7	54 9	56 3
	5	55 4	68 8	47 4		72 8	72 3	73 0	-
	6	54.8	36 8	73 9[a]	54 1	87 0	79 8	82 9[a]	71 1
	7	58 3	52 4	70 9[a]	80 7	77 1	80 8	82 0[a]	69 6
	8	48 8	50 1	68 5	53 0	70 9	60 3	70 8	59 0
	9	51 2	53 2	62 4	56 3	69 7	69 0	73.4[a]	52 9
	10	74 4	86 3	72.3	85 2	81 0	75 9	77.4	66 5
	11	50 5	73 2	72.4	74 5	73 5	67 2	75 2	58 2
	12	65 2	74 8	47 9[a]	53 9	73 9	74 9	81 5	54 1
	13	59 8	75 0	-	56 9	88 9	81 0	-	77 8
	14	(67 7)				(77 9)			
	15	(78 1)				(73 1)			
	16	(.0 6)				(77 1)			
	17	(68 7)				(63 2)			
	X̄	60 9	65 4	67 1	66 8	75 3	70 5	72 9	61.2
	SD	9 2	15 9	12 9	12 9	7 5	7 7	8 8	8 2
	n	12	12	11	11	13	13	12	12
Diff rence		18 8	17 2	22 7	20 6	21 1	-18 1	18 3	-14 1
P		xx	x	x	x	xxx	xxx	xx	x

[a] Exercise terminated afte 10-18 minute because f fatigue

Tabl I. Heart rate (beats/min) at rest (RI) at 10 and 15 minute and at the end of two generally 20-minute periods of supine leg exercise (WI and WII) and t 5 10 20 and 30 minutes after exercise (RII) in the patients and the controls Data at rest are given for 4 controls in whom blood flow values during exercise had to be discarded Figure within parentheses are not included in mean values Other symbols a in Table IV

	RI	WI			WII			RII			
		10	15	20	10	15	Final	5	10	20	30
Pati nt no 1	68	110	110	112	140	140	140[a]	110	108	75	75
2	73	125	125	125	145	155	160[a]	120	105	100	95
3	71	90	96	93	110	115	120	100	95	90	90
4	93	120	120	120	160	160	160[a]	120	125	120	120
5	77	130	126	135	158	180	180[a]	118	110	113	95
6	47	110	118	130	165	170	180[a]	110	100	-	80
7	63	118	118	113	170	170	174	110	102	96	92
8	87	129	128	140	173	177	178	122	116	110	106
9	82	122	122	123	167	168	170[a]	119	118	108	94
10	91	164	170	168	190	-	194[a]	130	125	122	118
X̄	75	122	123	12?	158	159	166	116	110	104	97
SD	14	19	19	20	22	21	22	8	10	15	15
n	10	10	10	10	10	9	10	10	10	9	10
Control no 1	53	112	112	116	172	175	177[a]	96	90	75	67
2	68	108	116	116	178	184	188[a]	110	102	97	93
3	63	108	108	114	(185)		185[a]	115	111	97	90
4	42	102	100	100	147	156	168	90	84	80	65
5	60	117	118	118	169	176	181[a]	114	108	100	87
6	56	117	118	118	(193)	-	193[a]	116	104	89	82
7	70	125	130	135	170	170	175[a]	103	102	90	90
8	60	120	120	125	160	160	165[a]	110	94	85	85
9	74	124	124	136	172	175	180[a]	120	98	88	86
10	61	84	86	90	132	138	140	76	76	76	70
11	59	108	108	111	161	165	170[a]	100	91	86	81
12	61	121	121	126	(192)	-	192[a]	110	110	108	103
13	61	-	100	104	146	(161)	161[a]	94	88	82	78
14	(65)										
15	(56)										
16	(74)										
17	(63)										
X̄	61	112	112	116	161	167	175	104	97	89	83
SD	8	11	12	13	15	14	15	13	11	10	11
n	13	12	13	13	10	9	13	13	13	13	13
Diff rence	14	10	11	10	-3	-8	-9	12	13	15	14
P	xx	>0 10	>0 10	>0 10	>0 70	>0 30	>0 20	x	xx	xx	x

[a] Exercise termi ted aft 10-18 minutes because f f tigue

Acta Medica Scandinavica

Supplementum 563

Neues über die Wirkungsweise der menschlichen Kohlenmonoxidvergiftung und über die Sauerstoff-Bindungsfähigkeit menschlicher Erythrozyten

Neues klinisches Frühsymptom der Co-Vergiftung

Von K. Lenggenhager

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

Neues uber die Wirkungsweise der menschlichen Kohlenmonoxidvergiftung und über die Sauerstoff Bindungsfähigkeit menschlicher Erythrozyten

Neues klinisches Frühsymptom der CO Vergiftung

INHALT

I SUMMARY

A number of clinicians attribute a specific effect to carbon-monoxide intoxication in man. Meldrum and Roughton for instance have demonstrated inhibition of carboanhydrase by CO in test tube experiments.

Should this view prove valid, one would expect that recumbent test persons with moderate CO-saturation would develop a considerably diminished tendency to hyperventilation tetany—owing to the inhibition of carboanhydrase. However, in a group of 12 normal persons subjected to 25–30% CO-blood-saturation, a series of 6–10 deep breaths in very rapid succession was regularly followed by brief unconsciousness.

This phenomenon cannot be fully explained either by Haldane's CO–O_2-blood saturation curves or by the displacement of the BOHR curves to the left due to the loss of CO_2. Attention is called to the misleading presentation of the CO–O_2-binding curves of blood in Haldane's work. Even in the presence of varying degrees of CO-presaturation all the curves derived from complementary oxygen saturation are plotted against the zero mark of the same curve and not—as might be expected—against values corresponding to, for instance, the 25, 50 or 75% presaturation levels chosen in the investigation.

In CO-intoxication of man, carbon monoxide appears at the upper end of the saturation curve because at this level the least amount of resistance is offered to displacement of oxygen.

Correspondingly, CO breathing at rest at high altitudes does not cause symptoms as long as the uppermost, oxygen-free part of the O_2-saturation curve alone is taken up by carbon monoxide. Only when CO-saturation is augmented and oxygen is progressively displaced from the erythrocytes do symptoms of tachycardia and drowsiness occur.

A new discovery is the fact that the onset of hyperventilation-coma occurs earlier at high altitudes than at sea-level in the presence of constant levels of CO-blood saturation. This can be attributed to a specific effect of alcalosis upon the brain.

This effect is enhanced by the strong left shift of the O_2-saturation curve caused by hyperventilation. As measured by modern spectrographic methods, this curve also shows a progressive upward trend.

However, neither the rectification of the curves nor the modification of O_2-binding due to alcalosis suffices to explain fully the phenomenon of hyperventilation coma.

It was shown that in blood which becomes progressively deficient in CO_2 the binding curve for O_2 moves gradually to the left over the whole scale, and not mainly in the medium range. This too has been determined by modern spectrographic CO-oxymeter measurement.

Thus we are able to understand the mechanism of our experiment in which maximum hyperventilation leads to coma even when blood pressure remains unchanged. For ultimate comprehension of this form of coma and the loss of consciousness brought about by 25% CO-blood-saturation and brief hyperventilation, the following is of importance: cerebral function, mainly as concerns the conscious state, requires a certain elevation of the minimum oxygen tension level in human beings who are not adequately trained or accustomed to alcalosis.

Full understanding of hyperventilation coma in slightly CO-intoxicated persons results from a combination of the following three elements:

1. Reduction of the amount and tension of O_2 in Co-blood.
2. Reduction of tension of O_2 due to alcalosis.
3. Elevation of the minimal O_2-tension threshold necessary for the state of consciousness, owing to a detrimental effect of alcalosis.

These new views offer a better explanation for the drastic differences of behaviour in patients with anaemia in the range 50–30% hemoglobin who are still capable of work; this stands in contrast to a 50% CO-blood saturation, which represents the utmost level compatible with the state of conciousness at complete rest and only in a recumbent position.

Finally, it has been shown in animal experiments that CO mainly affects the breathing centre, rather than the heart. This is why these animals with paralysed respiration recover under O_2 over pressure.

Das Internationale Symposium über Kohlenmonoxydvergiftung (Mai 1965) wurde vom Vorsitzenden Symanski mit den Worten eröffnet. „Der Überblick über Schrifttum und Praxis zeigt dass immer wieder unzutreffende Vorstellungen über das Wesen und die Folgeerscheinungen der CO-Vergiftung bekannt werden

Claude Bernard entdeckte 1857 die grosse Affinität des Kohlenmonoxyds zum Haemoglobin und erklärte den CO-Tod als innere Erstickung was jedoch wegen der zu gross scheinenden Verschiedenheit zwischen O_2-Mangel und CO-Intoxikation stark bezweifelt wurde

Haldane (1895 und 1896) fundierte die Erklärungen Bernards über die Wirkungsweise der akuten CO-Vergiftung, später auch anhand eindrücklicher Selbstversuche Für ihn waren ebenfalls alle Symptome durch den teilweisen Beschlag der Erythrocyte mit CO und der daraus resultierenden zwangsläufigen Hypoxie der Gewebe erklärt.

Während diese Theorie im Verlauf durch die Physiologen wohl allgemein Anerkennung fand stiess sie bei den Klinikern zum grossen Teil auf hartnäckigen Widerstand, indem die Symptome bei CO-Vergiftung zu verschieden und viel zu mannigfaltig scheinen gegenüber den reinen Symptomen blossen O_2-Mangels worauf unter anderem auch Zorn Strabafin, Moeschlin Claude Mosinger u a. hinweisen. Da ich selbst auch der Gruppe von Zweiflern angehörte unternahm ich zahlreiche Tier und Eigenversuche bestrebt irgend etwas spezifisch Toxisches für die CO-Vergiftung herauszufinden. Ich möchte vorwegnehmen dass mir letzteres nicht gelungen ist, so dass ich jetzt ebenfalls zum überzeugten Anhänger der Haldane schen Auffassung geworden bin allerdings mit einer neuen wesentlichen Modifikation weshalb mir die Veröffentlichung und die etwas längere Einleitung dieser Arbeit gerechtfertigt erscheinen.

Ausgangspunkt bildete zunächst folgende Fragestellung Warum werden Mensche bei protrahierter Atmung o O_2-armer Luft häufig tet nisch oder praetetanis h ährend dies bei de CO-Vergiftung kaum vorzukommen schei t oder jedenfalls seh selten ist (Zit nach H nderson aus dem Handbuch der Speziellen pathologischen Anatomie und Histologie 1958) Wohl abe finden sich bei de CO-Vergiftung kypotische Krämpf und echs lnde klonisch-tonische Rigorzustände

Könnte also die praktisch fehlende Tetanie bei der CO-Vergiftung ev auf Hemmung der Carbanhydrase durch CO beruhen wie dies Meldrum u Roughton (1934) glauben?

In einem Eigen ersuch wurde ich in einer Unterdruckkammer entsprechend einer Höhe von 9 000 m ü M ohne Benützung von Sauerstoff tetanisch (Diesen Versuch, der 45 Minuten dauerte verdanke ich Herrn PD Dr F on Tavel welcher in seiner Unterdruckkammer die Fliegereignungsprüfungen leitete) Durch Atmen von CO_2 ging die Tetanie jeweils zurück um wenige Minuten nach Sistieren der CO_2-Atmung infolge des vergrösserten Atemvolumens wiederum zu erscheinen. Hier hatte also der O_2-Mangel in der Atmungsluft genügt, um über den Weg erstärkten Atmens zu einem konsekutiven CO_2-Verlust zu führen. Nun aber bewirkt die Anwesenheit des Fermentes Carbanhydrase dass (bei ermehrtem Abrauchen von Blut-CO_2) aus dem physiologischerweise im Blute vorkommenden Natriumbikarbonat ($NaHCO_3$) unter Abspaltung von CO_2 das stark alkalisch reagierende Na-Karbonat (Na_2CO_3) entsteht Letzteres führt aber nach eigenen Untersuchungen über den Weg verminderter Calciumionisierung zur Tetanie (Lenggenhager 1951) So findet man nach kürzerer aber stärkster Hyperventilation schon Verschiebungen des Blut-pH um 0 15–0,25 nach der alkalischen Seite! Durch blosses Abatmen von gelöster CO_2 ohne die Carbanhydrase wäre nach chemischer Vorstellung nicht mit einer merkbaren Änderung der Reaktion (pH) zu rechnen Auch von Schroetter (1904) Fleming (1911) und Barcroft (1927) fan-

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Während 16 Jahren (1942–1960) haben wir während der Frühlingsferien jewells 14-tägige höhenphysiologische Studien im Forschungsinstitut auf Jungfraujoch (3 450 m) mit je 1.–14 Teilnehmern durchgeführt. Ein kleiner Bruchteil der Ergebnisse findet in dieser Arbeit seinen Niederschlag.

den in grossen Höhen tetanische Erscheinungen (zit. bei Loewy S. 767.)

Umgekehrt sind also bei CO-Vergiftung praktisch keine rein tetanischen Zustände (wohl aber hypoxische Krämpfe) bekannt.

Damit schien aber klinisch eine gewisse Möglichkeit zu bestehen, anhand dieser Carbanhydrase-Hemmung durch CO eine allfällig verzögert auftretende Hyperventilationstetanie im Eigenversuch zu erhalten, verglichen mit vorgängigen Hyperventilationstetanie-Zeiten vor der CO-Atmung. Da (durch eigene Methode) mein Schüler Stirnemann eine Teilentionisierung des Blutcalciums während Hyperventilationstetanien nachweisen liess (unter Konstanz des absoluten Ca-Gehaltes), wollten wir also eine verminderte Calcium-Teilentionisierung bei CO-Versuchspersonen im Hyperventilationstest nachweisen.

Ruhe-Hyperventilation bei CO-Vergiftung

Nachdem ich während 35 Minuten ein 5‰iges CO-Luftgemisch in liegender Lage geatmet hatte, betrug meine Blut-CO-Sättigung 30% (Die Blut-CO-Bestimmungen wurden im Physiologisch-Chemischen Laboratorium durch Prof. J. Abelin, Ordi- für physiologische Chemie, persönlich aus-) Der Ruhepuls war von 60 auf 76 ge- iegen, die Gesichtshaut gerötet. Es bestanden Zeichen von ganz leichtem Schwindel, Euphorie und Dösigkeit.

Jetzt begann ich liegend gegen gewöhnliche Zimmerluft maximal aktiv zu hyperventilieren in der Vorstellung, die sonst bei mir so typische Überatmungstetanie nicht mehr (oder stark verspätet) zu erhalten. *Wir erstaunt waren wir alle, als ich schon nach de ersten 8 tiefen Atemzüge welche i nerhalb von 10 Sekunden erfolgten, plötlich und oh e jegliche Vorboten bewusstlos wurde und Krämpfe und Zuckungen bekam.* Nach ungefähr 10 Sekunden war ich wiederum bei Bewusstsein.

I h w e lt den Versuch. Es genügten sogar 6 tief d emzüge um ca 3 Sekunden später w ch rubergehende Bewu st losigkeit w um K mpfen zu erzeugen. Ein Hyper h m t reinem O_2 f hrte dagege t S kunden zu den gleichen Erschei nge

I meiner M t bei ebenfall zu diesem Versuche ber l wurde das gleiche Phänomen e wiese ei en

Blut-CO-Gehalt on 28–35% auf. Alle mussten ebenfalls ca 35–40 Minuten lang ein 5‰iges CO-Luftgemisch liegend atmen, bis die 30%ige Blut-CO-Sättigung erreicht war. (Insgesamt 18 Versuche mit 12 Versuchspersonen.)

Sofort anschliessende gleichstarke Hyperventilationen derselben CO-Versuchspersonen mit einem 5%igen CO_2-Luftgemisch machten während 3–5 Minuten keinerlei pathologische Symptome (es wurde nicht länger geprüft). Dagegen stellten sich bei absichtlicher weiterer reiner Luft-Hyperventilation die beschriebenen, schweren Zustände wiederum ein. Mit zunehmender Wartezeit (in Liegelage) konnten diese Hyperventilations-Ohnmachten zwar noch nach 1 Stunde ausgelöst werden, bedurften aber einer etwas längeren Hyperventilation von 15–18 Sek. Dauer. Die Dauer dieser kurzen, mit klonischen Zuckungen einhergehenden Ohnmachten betrug je nach Versuchsperson 10–20 Sek.

Es mag zunächst überraschend erscheinen und hat auch uns erblüfft, dass es zur Erreichung on nur 30–35% Blut-CO-Sättigung eines so langen Atmungsversuches mit so hoher 5‰iger CO-Beimischung bedarf, während in den toxikologischen Büchern (z.B. Flury nd Zernik (1931), Teleky (1955) und andere) diese Konzentration als tödliche Grenze angegeben wird. Nach Teleky führt Einatmung von 0,1% CO-Luft zu 51,5%iger Blut-CO-Sättigung nd damit zu Synkope. Es hängt dies sicher damit zusammen, dass wir ausgeruhte, *lieg nde* Versuchspersonen mit minimalem Ruhe-Atemvolumen waren. Im Gegensatz hierzu befällt die CO-Vergiftung des Alltagslebens werktätige Menschen mit grösserem Atemvolumen. Auch die Selbstversuche durch J. S. Haldane (1895) wurden im Sitzen ausgeführt. Es genügte hierbei schon eine CO-Beimischung von nur 0,4‰ zur Erzielung einer Blut-CO-Sättigung on 30%, wobei (wohl wegen des Sitzens) eine Vergrösserung des Atemvolumens erwirkt wurde. Allerdings dauerten die Einatmungsversuche dann ein Vielfaches nserer ca. halbstündigen Einatmungsversuche.

Als Nebenbeobachtung sei erwähnt, dass unsere Minutenatemvolumina nur während des mittleren Drittels dieser Versuche mit CO-Einatmung leicht ergrossert wurden. Im Durchschnitt blieben die Atemvolumina in der Norm. E soll später noch näher darauf eingegangen werden.

Damit abe haben ir mit unse em Hyperventilationstest in liegender Ruhelage vermutlich ein Früh mptom leicht CO- ergift g ge- f nd die a sserordentlich rasch a ftrete de a h or berg h nd mit K ämpfen erbundene Ohnmacht na h chon 6 tiefst raschen I ge-

wöhnlicher Luft erfolgenden Atemzügen in Liegelage (in je 1 Sek. 1 tiefste In- und Exspiration!)

Nach Zorn ist sonst leichte CO-Vergiftung nur über den Weg mühsamer Blutgasanalysen erfassbar. *Der beschriebene Test füllt damit eine Lücke in der Früherfassung leichter CO-Vergiftung en.*

Diese Zustände sind nach eigenen Untersuchungen genau gleich wie die durch bewusste Hyperventilation in reinem Stickstoff sich so bald einstellenden hypoxischen Krämpfe. Sie müssen ebenfalls als hypoxisch bedingt aufgefasst werden, da sie bei Hyperventilation gegen reinen O_2 erst später auftraten: Durchschnittlich in 80–120 Sekunden gegenüber 8–15 Sekunden bei Lufthyperventilation. Das Erstaunliche dabei aber war die Tatsache, dass die Ohnmacht also selbst bei reiner O_2-Hyperventilation noch auftrat. Auch dieses regte zur weiteren Erforschung mächtig an.

Der Unterschied, warum Haldane im Sitzen trotz einer Blut-CO-Sättigung von rund 30 und mehr Prozent durch die hierbei leicht vergrösserte Atmung diese eben beschriebenen O_2-Mangelkrämpfe nicht bekam, beruht auf dem im Sitzen nur leicht gesteigerten Atemvolumen und der durch die Muskelaktion im Sitzen vermehrt gebildeten Muskelmilchsäure. Desgleichen konnte ich 15 Minuten nach Beendigung eines 35 minütigen CO-Atmungsversuches (von 5‰ CO in der Atmungsluft) zwar unter grosser Atemnot in einem Fahrrad lange Zeit herumfahren, ohne jedoch die Hyperventilationsanoxie-Anfälle zu bekommen, welche sich bei Hyperatmung im Liegen so prompt einstellen.

Durch die Säuerung und die hierdurch bedingte grosse Atmung wurde ein Grossteil des Blut-CO ausgetrieben, sodass nach dieser Leistung subjektives Wohlgefühl empfunden wurde mit Ausnahme leichter Kopfschmerzen.

Aus den beschriebenen Hyperventilationscomazuständen bei nur 25%iger CO-Blutsättigung ergibt sich immerhin die Tatsache, dass durch eine 30%ige CO-Blutsättigung jedenfalls die Carbanhydrase in keiner Weise gehemmt war.

Zudem ergaben spätere Untersuchungen meines Schülers Schlapfer, dass erst durch 50%ige CO-Konzentration in Luft (entsprechend einem Druck von ½ Atmosphäre) eine erste leichte Hemmung der Carbanhydrase feststellbar wurde, gemessen an der resultierenden Alkalität einer während 20′ in Zimmerluft gestandenen 0,1%igen $NaHCO_3$-Lösung mit und ohne Carbanhydrasezusatz. Bedenkt man aber, dass es zur schweren CO-Vergiftung des Menschen weniger als 1 mmHg CO-Druck braucht, so ergibt sich die riesige Überdosierung des CO im erwähnten Versuch mit seinem ungefähr 360 mmHg betragenden CO-Druck (bei einem Barometerdruck von 720 mm). Man bedenke hierbei, dass schon ein um nur das 3–4fache gesteigerter O_2-Druck innerhalb weniger Tage für den Menschen und Säugetiere tödlich ist. So starben z.B. unsere 10 Meerschweinchen, die wir auf Jungfraujoch reinen O_2 bei einem Barometerdruck von nur 490 mm atmen liessen im Verlauf von 6 Tagen, während die Kontrolltiere, die Jochluft atmeten, gesund blieben. So begreift man auch, dass eine Druckerhöhung des schädlichen CO um mehr als das 360-fache noch schädlicher sein dürfte.

Die prompte Wirkungsweise unserer äusserst kurzen Hyperventilationsversuche bei nur mittleren Graden von Co-Vergiftung (alle Versuchspersonen waren gehfähig und schrieben fehlerfrei) weist aber nun, wie mir scheint, mit besonderer Deutlichkeit und vermutlich neuem Tatsachenmaterial in eine neue Richtung für die Erklärung der CO-Vergiftung.

Haldane' Erklärung der CO-Wirkung

Nach eigenen Versuchen von Haldane ging es zunächst darum, zu erklären, warum der menschliche Körper mit nur 50% CO-Gehalt seiner Erythrocyten schwerst geschädigt ist, nahe an der Grenze des Bewusstseins lebt und bei kleinsten taumelnden Arbeitsversuchen zu Ohnmacht neigt (vergl. dazu den Haldane'schen Eigenversuch mit 56%iger Blut-CO-Sättigung). Im Gegensatz hierzu werden chronische Anaemien von 50% Hb und weniger erstaunlich gut vertragen.

An mir selbst habe ich die *Haldane*'schen Angaben voll bestätigen können, indem ich bei 55%iger Blut-CO-Sättigung mehrmals bei Gehversuchen kollabierte und vorübergehend jeweils bewusstlos wurde. Dabei hatte ich jedesmal das einer orthostatischen Ohnmacht vorausgehende unangenehme Übelkeitsgefühl.

In Liegelage mit passiver Beinhochlage erholte ich mich jeweils rasch und konnte zwar zitterig, aber fehlerfrei schreiben wie z.B. in Abb. 1.

Zur Erreichung dieser hohen Blut-CO-Sättigung musste ich ein 7,5%iges (!) CO-Luftgemisch während 22 Minuten in liegender Lage einatmen.

Im grossen Gegensatz hierzu weiss man aber, dass ein 50% ger Haemoglobinverlust bei chronischer Blutarmut sich im Alltagsleben kaum auswirkt (erst bei grösserer Arbeitsleistung).

Abb. 1. Eigene Schriftproben während Atmung von 5% (?) CO. Selbst nach 33minutiger Atmung noch keine Schrift- und Rechenfehler, ... zittrige Schrift. Blut-CO-Gehalt 35%. Selbst nach 55%iger CO-Blutsättigung war die Schrift noch in gleicher Weise erhalten bei 7,5%iger CO-Atmung

Douglas und Haldane's Erklärung hierzu ist folgend: Durch teilweise Beschlagnahme der Erythrocyten durch das 50–300 mal grössere Affinität aufweisende CO werde die CO-Bindungskurve der Erythrocyten entscheidend geändert. So würde man zunächst erwarten, dass ein zu 50% durch CO ... Erythrocyt ... O_2-Aufsättigung ... Widerstand entgegen ... 50% ... 10 mmHg ... [illegible]

einfach grösser als 0.43 mmHg) O_2 in solche Erythrocyten aufgenommen werden kann und sogar in zunächst vermehrtem Masse. Im oberen Teil der Sättigungskurve dagegen geschehe umgekehrt eine Erschwerung der O_2-Bindung, sodass die Endaufsättigung durch O_2 wiederum bei normalen Spannungen erfolge (s. Abb. 2).

Dies führte Haldane zur Ansicht, dass im CO-haltigen Erythrocyten die O_2-Spannung eine normale sei, nur die Menge des gebundenen O_2 sei verringert (s. auf S. 241–242 seines Buches „Respiration").

Zur Kritik an der Haldane'schen Auffassung

Es mag wie eine Vermessenheit anmuten, wenn die Ansicht der beiden so verdienten und hervorragenden Forscher J. S. und J. B. S. Haldane hier einer Kritik unterzogen werden soll. Ich möchte meinerseits an dieser Stelle meiner allergrössten Hochachtung vor diesen vorbildlichen Forschern Ausdruck geben.

Nach Haldane nähme also ein teilweise CO-gesättigtes Blut zunächst leichter als normal O_2 auf, um dann umgekehrt bei höheren Drucken erschwert O_2 aufzunehmen, sodass die End-O_2-Spannung im Erythrocyten wieder normal hoch würde.

Besonders irreführend wären aber nach dieser Darstellung die Verhältnisse beim Vorliegen eines z. B. zu 95% vorgesättigten CO-Blutes. Hier müsste die O_2-Bindungskurve nach der Hal-

Pressure of O₂ in mmHg

Pressure of O₂ in % of an atmosphere

[illegible] erst hierte, später erschwerte [illegible] CO-Blut immer bei 40 mm [illegible] Haldane, Respiration S. 243) [illegible] CO-Blut III 35%iger CO- [illegible] IV [illegible] Blut V 75%iger CO-Blut

dane schen Darstellung zuerst nahezu senkrecht ansteigen, um oben fast rechtwinklig und normal weit nach rechts hinüberreichend zu verlaufen.

Es ist dies aber meines Erachtens ein Trugschluss bedi gt durch di l der Abb 2 trot T ll-CO-Sättigung der Erythrocyten gleichbleibenden Masstäbe für die Darstellung der weiteren prozentualen Aufsättigungskurven durch O Ist jedoch ein Erythrocyt z. B mit 25 % CO vorgefüllt so darf die weitere Auffüllu g mit O nicht mehr bei „Null" der Abb 2 st rten sondern bei 25 % der Ordinate für ein 50 %iges CO-Blut bei 50 und für ei 75 %iges bei 75 usw

Alle Werte, die in der Abb 2 z. B auf der O_2-Bindungskurve der 50 %igen CO-Vorsättigung liegen müssen also immer reduziert werden durch den Wert (94–50)/94 wobei 94 das in der Haldane-kurve bei 100 mm O_2-Spannung erreichte Maximum der O_2-Sättigung (entsprechend der Abb 2) darstellt. Dieser Faktor ergibt 0 468.

Diese O_2-Sättigungskurve muss wegen der 50 %igen Vorfüllung durch CO also bei 50 % der Ordinate beginnen!

Für die 25 %ige CO-Kurve ergibt sich ein Reduktionsfaktor von (95–25)/95 was einem Wert von 0 731 entspricht. Diese Sättigungskurve der Haldane schen Abb 2 muss nach Reduktion ihrer Werte bei 25 % gestartet werden. Für die 75 %ige CO-Kurve endlich ergibt sich ein Reduktionsfaktor von (93–75)/93 was den Wert 0,21 ergibt Alle Messpunkte der Original-CO–O_2-Kurv des zu 75 % orgesättigten CO-Blutes (Abb 2) sollen also um 0,21 reduziert werden und ihr Anfang bei 75 % beginnen.

Jetzt ersehen wir i ein- und derselben Darstellung sowohl die Spannungsverhältnisse als auch die absoluten Mengen des an die CO-Erythrocyten gebundenen O_2 sofern die Fusspunkte dieser Kurven als horizontale Abscissen verlängert gedacht werden (Abb 3)

Würde jedoch eine 50 %ige CO-Vorsättigung des Blutes den 30 mm Ladedruck bedingen, welcher für eine 50 %ige O_2-Sättigung dieses Normalblutes nötig ist so konnte die weitere O_2-Sättigung *dieses 50 % gen CO-Blutes erst erfolgen bei* Drucken die höher sind al 30 mmHg. Dann erst w re die Haldane sche Vorstellung richtig, dass es ich m CO-Erythrocyten bei Totalaufsättigung mit O_2 um normale O_2-Spannungsverhältnisse handeln würde

Nun darf man aber den alleroberstеn O_2-Sättigungswerten in stark CO-haltigem Blut, welche nach der Haldane schen Methode be-

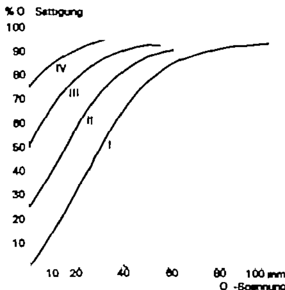


Abb 3 Zeigt die einem einheitlichen Masstab angepassten Sättigungskurven der Abb 2. I = Normal-Kurve der O_2-Sättigung von Blut II = 25 % CO-Vorsättigung, dann O_2-Nachsättigung. III = 50 % CO-Vorsättigung, dann O_2-Nachsättigung, IV = 75 % CO-Vorsättigung, dann O_2-Nachsättigung

stimmt werden kein grosses Gewicht mehr beimessen da diese Methode mit je nur 1–2 ccm Blut arbeiten muss weil durch grössere CO-Blutmengen nach der Gasaustreibung nach Haldane s Angaben eine Teilrückabsorption des ausgetriebenen Gases erfolgt. Infolge der überaus *kleinen* O_2-Volumina der ausgetriebenen allerletzten O_2-Prozente aus CO-teilgesättigtem Blut sind diese obersten Kurvenabschnitte eher spekulati Es wird später darauf eingegangen Damit erscheint die Ansicht einer normalen O_2-Endspannung in CO-haltigem Blut rein theoretisch.

Nun gibt es aber noch 2 Gründe welche gegen die Ansicht einer normalen O_2-Spannung im teilbelegten CO-Blut nach Aufsättigung mit O_2 bei normalem Luftdruck sprechen Erstens haben wir in unseren 12 Versuchen mit 8 Versuchspersonen welche ausgeruht im Liegen ein 5 %-iges CO-Luftgemisch atmeten schon nach 7 Minuten einen Pulsanstieg on 59 auf 67 bei noch völligem subjektivem Wohlbefinden festgestellt Dieses Frühwarnzeichen wäre unverständlich bei der Vorstellung einer normalen O_2-Spannung.

Zweitens wurde auch das Ruheminutenatemvolumen nach 7 Minuten und während der nächsten 21 Minuten in diesen Versuchen leicht

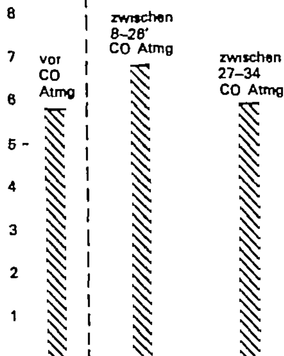


Abb. 4 zeigt, dass am Ende einer 34minütigen 5%igen CO-Atmung (Maskenatmung) das Atemvolumen nicht mehr erhöht ist. Durchschnittswerte von 5 Personen (Durchschnittsalter 29 Jahre).

erhöht (S. Abb. 4). Auch dies bedeutet eine bald auftretende Stressreaktion, die noch kaum subjektiv wahrgenommen wird. Wiederum wäre dies beim Vorliegen normaler O_2-Spannung nicht erklärt.

Als weiteren Test für die Richtigkeit dieser Überlegungen erblicke ich auch folgendes: zeichnet man die nach besprochener Weise reduzierte O_2-Sättigungskurve eines 50%igen CO-Blutes der Abb. 3 auf Seidenpapier, so lässt sich diese Kurve in nahezu idealer Weise mit der oberen Hälfte der normalen O_2-Sättigungskurve (Kurve I der Abb. 3 ...) Deckung bringen, sofern d.e reduz... K.rve bei 50% der normalen O_2-Sättigu... ...tet w.d. Hierbei muss die Kurve um ... ...erschoben werden, wobei jed... P... ...gehen mit der Normalkurve ... Sp... ...minderung ...on 30 mmHg aufwei...

Ähnliche V... ...ich aber auch mit der 25... ...Kurve. Offenbar benützte aber H... ...Versuche eine zu 28% mit CO vorgesättigte Blutprobe anstelle einer 25%igen, was aus der guten Überlagerungsmöglichkeit hervorgeht.

Dagegen ergeben sich aus der modifizierten 75% CO-Kurve nur noch andeutungsweise die obersten 25% einer O_2-Sättigungskurve. Dies ist wohl bedingt durch die erwähnte Tatsache der immer schwieriger werdenden Bestimmungen sehr kleiner O_2-Beträge im Endteil der Sättigungskurve, wenn man bedenkt, dass Haldane in seinem Apparat nur je 1(2) ccm Blut pro Analyse benutzen durfte.

Auch folgende Methode rechtfertigt die beschriebene Auffassung einer zu reduzierenden Darstellung der O_2-Bindungskurve eines teilweise mit CO vorgesättigten Blutes. Alle horizontalen Verbindungslinien zwischen Kurve I, II und III der Abb. 3 weisen (mit Ausnahme der alleroberste weil ungenaueren Abschnitte) nahezu überall den gleichen Betrag auf. Dieser beträgt für das 25%ige CO-Blut ca. 15 mm O_2-Spannung, für das 50%ige ca. 30 mm.

Damit ist also z.B. die O_2-Bindungskurve des 50%igen CO-vorgesättigten Blutes ganz einfach um den Wert von 30 mm Spannung nach links verschoben. Sie sättigt sich also überall bei um 30 mm verminderter O_2-Spannung auf ... verglichen mit der normalen Blutsättigungskurve (vergl. Abb. 5).

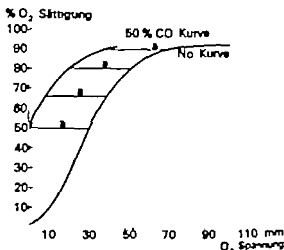


Abb. 5. Obere Kurve: O_2-Bindungskurve in 50% CO-Blut bei 40 mm CO_2-Spannung. Untere Kurve: normale O_2-Bindungskurve bei 40 mm CO_2-Spannung. Die Querabstände a bleiben sich gleich bis zu 90%iger O_2-Sättigung. Gleiche Sauerstoffsättigungen geschehen also in diesem CO-Blut praktisch immer schon bei um 30 mm niedrigeren O_2-Spannungen.

Man könnte diesen Überlegungen den Vorwurf blosser Spekulation machen Deshalb liess ich diese Frage durch meinen Schüler Spycher in einer Inauguraldissertation anhand des modernsten spektrografischen Instrumentes des „CO-Oxymeters M 182 der JL Inc Lexington Mass" überprüfen Das Instrument analysiert automatisch bei 38°C Eine peristaltische Pumpe saugt anaerob die Blutflüssigkeit auf hämolysiert die Probe und bringt sie in die Mess-Cuvette Über den Weg der spektroskopischen Absorption registriert das Gerät simultan die Blutprobe bei 3 sorgfältig ausgewählten Wellenlängen im grünen Spektralbereich Dies gestattet die prozentuale Feststellung des Oxyhämoglobins CO–Hb und des totalen Hämoglobins in ein und derselben Probe

Vorgehe fü die Herstellung der ge nschte Co–O_2-Blutsättig gskurven

Alle Versuche wurden im Wasserbad bei 38°C durch meinen Schüler Spycher durchgeführt (s Abb 6) Da die Blutgassättigung stark temperaturabhängig ist muss ein Thermostat mit Rührwerk für Temperaturkonstanz sorgen.

Damit infolge der relativ hohen Temperatur und des vorbeiströmenden, trockenen Gasgemisches kein Eindicken des Versuchsblutes eintritt wird das Gasgemisch durch eine dazwischen geschaltete Wasserflasche („2" der Abb 6) geleitet.

Der Schüttelkolben ist an eine Wasserstrahlpumpe angeschlossen die ein dosiertes Durchströmen des Kolbens ermöglicht und durch den Hahn B abgeschaltet werden kann. Hahn A ermöglicht das Einfüllen und Entnehmen der Blutproben.

Versuchsvorga g

1 ca 20 ccm heparinisiertes menschliches Frischblut, unmittelbar or Versuchsbeginn entnommen wird in einem Erlmeyerkolben von 100 cc durch Schütteln n ständig zugeführten, reinem N reduziert

2 Herstellung der Gasgemische:

a) für CO-Sättigung des Blutes. In einem evakuierten 200 l fassenden Gummisack werden total 150 l gem scht

Herrn Prof Dr H P Gurtner Leiter der kardiologischen Abt. des Inselspitals sei auch an dieser Stelle herzlich gedankt für die Erlaubnis, mit diesem Apparat arbeiten zu dürfen.

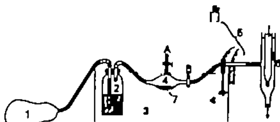


Abb 6 1 Gasgemisch von 150 l in Gummisack *2* Wasserflasche zur Konstanthaltung der H_2O-Spannung: *3* Wasserbad von 38°C *4* Schüttelkolben von 200 cc Inhalt mit jeweils 4 cc Blut; *5* Heizung durch Thermostat mit Rührwerk *6* Wasserstrahlpumpe *7* Blut, *A* = Hahn zur Blutentnahme: *B* = Hah für Gasfüllung des Schüttelkolbens *4*

150 mm Spannung CO 40 mm Spannung CO_2 werden ergänzt auf 150 l mit reinem, O_2-freiem Stickstoff

Mit diesem Gasgemisch werden zunächst ca 20 cc heparinisiertes Blut bei 38° entspr d Abb 6 öllig CO gesättigt Kontrollmessungen im CO-Oxymeter ergaben 100 % CO-Sättigung.

b) In einem 2. Gummisack werden Gasgemische der gewünschten O_2–CO_2-Spannung nach folgender Formel hergestellt.

$$\frac{\text{gewünschte Spannung von } O_2 \text{ oder } CO_2}{\text{jeweiliger Barometerstand am Versuchstag}} \quad \frac{\text{z. B} \quad 50\ \text{mmHg}}{\text{z B} \quad 720\ \text{mmHg}} \quad \frac{x\ \text{l}}{150\ \text{l}} \rightarrow x$$

$$= \frac{150 \cdot 50}{720} = 10\ 416\ \text{l}$$

3 *Beispiel für ein 50 %iges CO–Blutgemisch mit nachfolgenden O_2-Teilsättigungen bei 40 mm CO*

Bei 38 wird zuerst mittels Wasserstrahlpumpe aus dem mit dem jeweiligen O_2–CO_2 Gasgemisch vorbereiteten Gummisack das gesamte System der Abb 6 vorgefüllt. Hernach wird Hahn B geschlossen. Der Gummisack wird nun so unter Druck gesetzt dass beim Öffnen des Einfüllhahnes A der Abb 6 nur Gas entweichen, aber keine Aussenluft eindringen kann.

Mittels Spritze und feinem Katheter werden 2 cc CO-Blut und sofort 2 cc reduziertes Normalblut eingefüllt. Nun wird 10' lang abwechslungsweise das Blut kräftig in dem Kolben geschüttelt (wobei

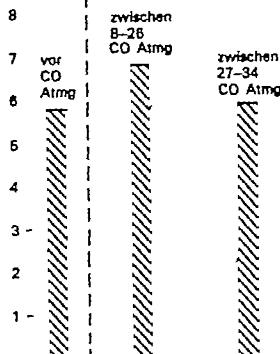


Abb 4 zeigt, dass am Ende einer 34minütigen 5%igen CO-Atmung (Maskenatmung) das Atemvolumen nicht mehr ergrössert ist. Durchschnittswerte von 5 Personen (Durchschnittsalter 29 Jahre).

erhöht (S Abb 4) Auch dies bedeutet eine bald auftretende Stressreaktion, die noch kaum subjektiv wahrgenommen wird. Wiederum wäre dies beim Vorliegen normaler O_2-Spannung nicht erklärt

Als weiteren Test für die Richtigkeit dieser Überlegungen erblicke ich auch folgendes, zeichnet man die nach besprochener Weise reduzierte O_2-Sättigungskurve eines 50%igen CO-Blutes der Abb 3 auf Seidenpapier so lässt sich diese Kurve in nahezu idealer Weise mit der oberen Hälfte d[illegible] normalen O_2 Sättigungskurve (Kurve I der Abb [illegible] zur Deckung bringen sofern die reduzier[illegible] CO-Kurve bei 50 % der normalen O_2-Sättigun[illegible] ge[illegible] rd H erbei muss die Kurve um [illegible] m nach [illegible] verschoben werden wobei jed[illegible] le Punkt [illegible] nen mit der Normalkurv[illegible] Spannu[illegible] derung on 30 mmHg aufw[illegible] n

Ähnliche V[illegible] ältnisse [illegible] b aber auch mit der 25 % [illegible] O_2 B[illegible] [illegible] Offenbar benützte aber H[illegible] lane [illegible] be eine zu 28 % mit CO vorgesättigte Blutprobe anstelle einer 25%igen was aus der guten Überlagerungsmöglichkeit hervorgeht.

Dagegen ergeben sich aus der modifizierten 75% CO-Kurve nur noch andeutungsweise die obersten 25% einer O_2-Sättigungskurve. Dies ist wohl bedingt durch die erwähnte Tatsache der immer schwieriger werdenden Bestimmungen sehr kleiner O_2-Beträge im Endteil der Sättigungskurve wenn man bedenkt, dass Haldane in seinem Apparat nur je 1(–2) ccm Blut pro Analyse benutzen durfte.

Auch folgende Methode rechtfertigt die beschriebene Auffassung einer zu reduzierenden Darstellung der O_2-Bindungskurve eines teilweise mit CO vorgesättigten Blutes. Alle horizontalen Verbindungslinien zwischen Kurve I II und III der Abb 3 weisen (mit Ausnahme der allerobersten, weil ungenaueren Abschnitte) nahezu überall den gleichen Betrag auf Dieser beträgt für das 25%ige CO-Blut ca 15 mm O_2-Spannung, für das 50%ige ca 30 mm.

Damit ist also z B die O_2-Bindungskurve des 50%igen CO-vorgesättigten Blutes ganz einfach um den Wert von 30 mm Spannung nach links verschoben. Sie sättigt sich also überall bei um 30 mm verminderter O_2-Spannung auf verglichen mit der normalen Blutsättigungskurve (vergl Abb 5)

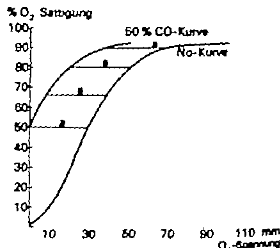


Abb 5 Obere Kurve O_2-Bindungskurve in 50% CO-Blut bei 40 mm CO_2 Spannung. Untere Kurve normale O_2-Bindungskurve bei 40 mm CO_2 Spannung. Die Querabstände a bleiben sich gleich bis zu 90%iger O_2-Sättigung. Gleiche Sauerstoffsättigungen geschehen also in diesem CO-Blut praktisch immer schon bei um 30 mm niedrigeren O_2-Spannungen.

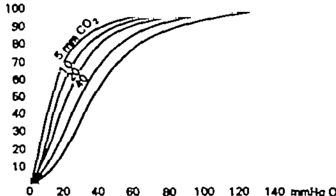


Abb 7 Prozentuale O_2-Sättigungskurven von Blut bei 38°C und verschiedenen CO_2-Spannungen (nach Bohr Hasselbalch und Krogh)

kurve nach links verschoben, im Sinne der leichteren O_2-Bindung ans Hämoglobin (s. Abb 7) Dadurch werden dem Gewebe rund 10 mm O_2-Spannung entzogen, nd die neue O_2-Sättigungskurve identifiziert sich nahezu mit der 50% CO-O_2-Kurve. Der resultierende Zustand entspräche nun also einem zu 50% mit CO vorgesättigten und dann durch O nachgefüllten Blut entsprechend der Kurve IV der Abb 2 im Zustande ohne Hyperventilation wobei jedoch noch keine Bewusstlosigkeit im Liegen auftritt. Dadurch w rd aber keine Erklärung gegeben für das so prompte Koma bei kurzer Hyperventilation im Zustand eines nur 25%igen CO-Blutes

Ein Versuch zur Deutung des Hyperventilationskomas anhand der reduzierten CO-O_2-Bindungskurven entsprechend der Abb 3 misslingt ebenfalls, indem durch hyperventilatorische Alkalose die nach links erschobene Kurve II der Abb 3 nicht einmal die Kurve III erreicht. Diese aber entspricht dem Zustande eines zu 50% CO-beladenen Blutes, was noch kein Koma im Liegen bedingt. Diese Linksverschiebung der O_2-Bindungskurven durch den CO_2-Verlu t (nach Bohr) sind m Abb 7 ersichtlich.

Es müssen als zu Erklärung andere Uber legu g n g tellt erd n nd m nicht CO-vor behandeltes Blut (in den Haldane schen Ver suchen) für die restliche O_2-A fsättigung benützt d sondern mgekehrt ein nter normalem O_2-Druck an Luft orgesättigte Blut das einer progr ss n CO-Kon entration ausgesetzt urd

So werden die Verhältnisse bei der menschlichen CO-Vergiftung imitiert, indem bei zunächst normaler Blut-O_2-Spannung sich kleinste Drucke on CO einschleichen.

Hierbei aber ist meiner Meinung nach zu er warten, dass diese kleinen CO-Spannungen zuerst progressi den noch durch O_2 frei gebliebenen Teil der Sättigungskurve auffüllen also die „ober sten noch leeren Teile der Hb-Bindungskurve belegen wo noch kein O_2 eingetreten ist und infolgedessen dem CO-Eintritt noch kein Konkur renzkampf mit dem O_2 bevorsteht. Es erinnert diese Situation an den in der ganzen Naturwissenschaft gültigen Satz des „geringsten Zwanges (nach Le Chatelier Eggert, Carnot, Helmholtz) M t zunehmender CO-Spannung wird also der O_2 on oben her" und zwar progressiv schwerer ausgetrieben weil dieser nach nten immer stärker am Hämoglobin haftet. Dies ist in der Abb 8 für ein 25% CO-nachgesättigtes Lungenblut dargestellt. Haldane hat selbst solche Bestimmungen der CO-Bindungsfähigkeit von vorgängig durch O_2 on rund 1" einer Atmosphäre (also bei normalem O_2-Druck vorgesättigtem Blut) orgenommen. Er hat gefunden, dass jetzt die CO-Bindungskurven rechtwinkelige Hyperbeln er geben also ohne den sonst obligaten Knick einer bei Null startenden O_2- oder auch CO-Sättigung zu ergeben (s. S 165/66 in „Respiration). Dies ist aus den Haldane schen Originalkurven zu ersehen (s Abb 9) Das aber heisst (meiner Meinung nach) dass in Luft-O_2-vorgesättigtem Blut kleine CO-Drucke im *oberen* und nicht im untersten Teil der Hb-Sättigungskurve eintreten Dies geht aus den zuerst steil, dann flacher wer denden CO-Bindungskurven der Abb 9 hervor weil die ersten CO-Mengen im oberen Ende der Hb-Sättigungskurve noch keinen Konkurrenzkampf mit dem O_2 ausfechten müssen.

Würde aber das CO zuerst am untersten Punkte

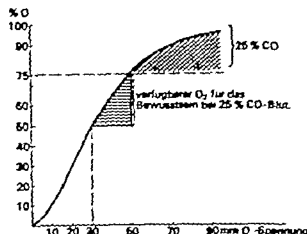


Abb. 8 Zeigt das zwar reduzierte aber immerhin noch genügende O_2-Produkt aus Menge mal Spannung.

der O_2-Bindungskurve eindringen, dann müsste die CO-Nachsättigungskurve auch die initiale Erschwerung aufweisen in Analogie zur gewöhnlichen CO-Hb-Sättigungskurve. Letzteres ist ja durch Haldane tatsächlich bestätigt worden in Analogie zur initialen Erschwerung der O_2-Aufnahme eines völlig O_2-frei gemachten Blutes (S. 165).

Haldane hat dieses nur so aufschlussreich erscheinende Ergebnis nicht für die menschliche CO-Vergiftung als Erklärung herangezogen, sonst hätte er auch zum Schluss kommen müssen, dass bei der CO-Vergiftung nicht nur die Menge, sondern auch die Endspannung des noch physiologisch bindungsfähigen O_2 im teilweise CO-belegten Blute verringert werden.

Für diese Auffassung, dass kleinste Mengen von CO das *oberste* Ende der O_2-Sättigungskurve beim lebenden Menschen belegen und deshalb praktisch subjektiv nicht empfunden werden, sprechen die Angaben im Prospect für das moderne CO-Oxymeter der Instrumentation Laboratory Inc. Lexington (Mass. USA). Danach finden sie bei Bewohnern grosser Städte, die Nichtraucher und symptomlos Blut-CO-Werte bis gegen 2 % vor, bei schweren Rauchern sogar bis gegen 10 %.

Demnach muss ma *h* *da* ***einem normalen Lun*** *d* *er* *Spa* *n* ***de eingeatmeten C(*** *he* *d* *ni ht* ***untersten) Ende der*** *B* *ku* *n-* ***dringen.***

Man könnte dieser ... CO-Eintrittes von der oberste ... H E ... kurve

her also von der umgekehrten Seite her' vorhalten, sie sei rein spekulativ. Zur Überprüfung dieser Frage haben wir jedoch an 5 Mitgliedern einer unserer Jungfraujoch-Expeditionen am ersten Tage des Höhenaufenthaltes (3450 m) in ausgeruhter Liegelage während 20 Minuten ein unreduziertes 4,5 %iges CO-Luftgemisch atmen lassen (was einem reduzierten Werte von 2,88 % CO entspricht), das während der ersten 10–12 Minuten im Gegensatz zu den Talversuchen ohne jegliche Änderung des subjektiven Befindens ertragen wurde. Die Blut-CO-Werte konnten dabei gestützt auf unsere zahlreichen Talversuche nur ungefähr 10–12 % betragen haben. Erst nach dieser Zeit geschah eine leichte Pulsfrequenzerhöhung von 64 auf 69/Min., als die Versuche nach 20 Minuten abgebrochen wurden. Wenn wir nun aber in Liegelage 6–8 tiefste Hyperventilationsatemzüge ausführten, so wurden alle 5 Versuchspersonen vorübergehend bewusstlos, genau so wie bei Tal-Hyperventilation bei 25–30 %iger Blut-CO-Sättigung. Auch für diese Versuche wäre die Erklärung nach der Haldane'schen Vorstellung nicht möglich. Sie ist aber wiederum die, dass das CO „von oben her" die Bindung mit dem noch freien Hb startet und sich deshalb zunächst nicht merkbar auswirkt, weil ohnehin die obersten 10–15 % des Hb auf Jungfraujoch-Höhe nicht durch den schwächeren O_2-Druck belegt werden, hier dringt

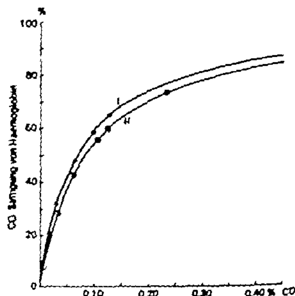


Abb. 9 Dissoziationskurven von CO-Hämoglobin bei konstant 20.9 % O und 38° Temperatur I=Blut von J. S. Haldane II=Blut von C. G. Dougla

das CO konkurrenzlos ins Hb ein. Da noch kaum O_2 verdrängt wurde, ändert sich nichts im subjektiven Bild.

Ganz ähnlich atmeten Douglas und Haldane im Tal ein Luftgemisch entsprechend einer Höhe von 5000 m. Sie machten die Beobachtung, dass eine CO-Vergiftung in diesem Zustand ohne subjektiv andere Symptome ertragen werden konnte, wenn die totale Blut–CO-Sättigung 23 % nicht überschritt (auf S. 279 des Buches von Haldane). Für mich wiederum ein Hinweis für restliche Auffullung der zu 2/3 mit O_2 vorgefüllten Erythrocyten durch CO, was für die O_2-Spannung keinerlei Abbruch bedeutet.

Bei Annahme der neuen Erklärung, nämlich der *herabgesetzten O_2-Spannung* und Menge eines menschlichen, vitalen, CO-teilbelegten Blutes ergeben sich nun auch geringere Differenzen beim Vergleich einer leichteren CO-Vergiftung mit der Höhenkrankheit. Haldane macht in seinem Buche „Respiration" auf die erhebliche Ähnlichkeit beider Zustände aufmerksam. Diese werden jedoch noch besser erklärt, wenn in *beiden* Fällen eine herabgesetzte O_2-Spannung der Erythrocyten vorliegt. Immerhin bestehen jedoch zwischen den Auffassungen von Haldane und zahlreichen Eigenuntersuchungen (s. Fussnote S. 1) mit vielen Versuchspersonen doch noch gewisse Unterschiede. So tritt in der Höhe nach Haldane und nach zahlreichen Eigenuntersuchungen (16 Expeditionen mit je 10–14 Versuchspersonen, je 14tägig im Forschungsinstitut Jungfraujoch (3 450 m) während der Frühlingsferien 1942–1960) doch noch gewisse Unterschiede. So tritt in der Höhe bei der Ruheatmung infolge der plasmatisch bedingten Herabsetzung der O_2-Spannung eine mässige Hyperventilation auf, was im Gegensatz zur praktisch normalen CO-Ruheatmung zu einer respiratorischen Alkalose des ersten Höhentages führt, auf welch letzteres auch Haldane hinweist.

Hinzu kommt also bei der Höhenatmung oder auch der stärkeren Tal-Hyperventilation noch der spezifische Alkaloseschaden, sogar in Form einer Überatmungstetanie bei starker Hyperventilation oder in grossen Höhen, welcher bei der CO-Vergiftung in der Ruhe fehlt. Letztere führt im Gegenteil zur Acidose, die Höhenkrankheit zu respiratorischer Alkalose. Allein auch diese neue Darstellung ergibt keine definitive Lösung des Problems, weil nämlich ein zu 50 % mit CO nachgesättigtes Lungenblut dann gar keinen O_2 mehr

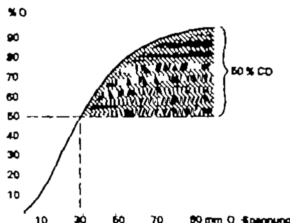


Abb. 10. Nach dieser Darstellung stünde dem Bewusstsein kein O_2 mehr zur Verfügung bei 50 %iger Blut–CO-Sättigung, was schon im Liegen zum Coma führen sollte, was jedoch nicht der Fall ist.

für das Bewusstsein zur Verfügung hätte, da die ganze obere Hälfte der Hb–O_2-Bindungskurve in Analogie zur Abb. 10 bis zur 50 %-O_2-Grenze hinab durch CO besetzt wäre. Dies aber würde einem völligen Bewusstseinsverlust gleichkommen. *Da jedoch ein Mensch mit 50 %iger Blut–CO-Belegung im Liegen noch durchaus nicht komatös ist, muss die Annahme gemacht werden, dass normalerweise der O in der Lungenluft bis auf mindestens 20 mm Spannung absinken kann, sofern der CO_2-Gehalt des Blutes nicht, wie in der Höhe, verringert wird,* sondern konstant bleibt oder sogar (wegen erlahmender Atemgrösse) leicht ansteigt. Diese Annahme wird im folgenden Abschnitt experimentell begründet.

Durch Berücksichtigung dieser Faktoren wird in Abb. 11 nun ersichtlich, dass trotz Vorliegens einer 25 %igen CO-Sättigung des Blutes intra vitam zwar noch ein recht erheblicher O_2-Vorrat zur Verfügung steht, dass jedoch bei kurzer Hyperventilation wegen des Bohreffektes (mit der Linksverschiebung der O_2-Bindungskurve) und der so schwerwiegenden Rechtsverschiebung der für das Bewusstsein verantwortlichen, minimal nötigen O_2-Spannung es zum Koma kommen muss. Die kleine obere O_2-Fläche der Abb. 11 ist eben unterschwellig für das Bewusstsein geworden. Als Vergleich seien die normalen O_2-Verhältnisse in einem Lungenblut in Abb. 12 dargestellt bei Innehaltung der CO_2-Spannung von 40 mmHg. Bei einem Atemanhalteversuch oder gar in einem schweren Asthmaanfall kann der O_2 dank der

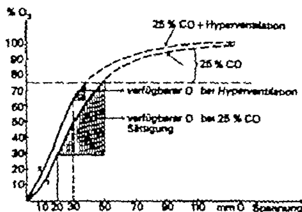


Abb 11 Die senkrecht schraffierte Fläche stellt das für das Bewusstsein verfügbare O_2-Produkt in einem mit 25% CO-teilgesättigten Vitalblut dar Die kleine horizontale schraffierte Fläche entspricht dem bei Hyperventilation resultierenden, zu Koma führendem zu kleinen O_2-Betrag.

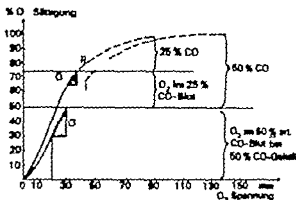


Abb 13 Kurve I Verhältnisse des intravitalen Blut-O_2-Angebotes bei 50%iger Blut-CO-Sättigung. Die schraffierte Fläche stellt das im 50%igen CO-Blut noch für das Bewusstsein zur Verfügung stehende zwar geringe Produkt aus O_2-Druck und O_2-Menge dar *Kurve II* stellt die Verhältnisse dar bei 25%iger Blut-CO-Sättigung und kurzer Hyperventilation Der O_2-Vorrat ist zu unterschwellig geworden: Koma

Acidose noch wesentlich tiefer im arteriellen Blut absinken So erinnere ich mich an eine 50-jährige Asthmatikerin, welche nach einer in Lokalanästhesie ausgeführten Operation einer stenosierenden Struma mit 2-stündiger Latenz in ein allerschwerstes Koma asthmaticum verfiel Trotz aller übrigen wirksamen Mittel blieb die Patientin während einer vollen Stunde tief bewusstlos in äusserster Cyanose Die Rettung brachte dann eine i v Injektion einer Ampulle Asthmolysin welche zu baldiger Normalisierung der Atmung führte Aus der Ohnmacht erwacht verblieb nur eine ca 3 Stunden lang dauernde Lähmung des rechten Armes mit nachfolgender völliger körperlicher und geistiger Restitution!

Nun kann aber ein Mensch mit 50%iger CO-Blutsättigung im Liegen und bei Normalatmung den O_2 dank normaler CO_2-Spannung in eben noch genügendem Masse ertragen Erst durch Mitberücksichtigung der alkalotisch bedingten Erschwerung der cerebralen O_2-Verwertbarkeit gelingt es unser eingangs beschriebenes Hyperventilationskoma bei nur 25%iger CO-Belegung des menschlichen Blutes zu erklären (s Abb 13)

Es soll deshalb noch näher auf den durch O_2-Mangel oder Hyperventilation bedingten Alkaloseeffekt eingegangen werden.

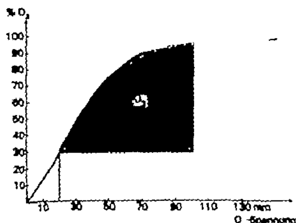


Abb 12 zeigt als Vergleich das Produkt aus Menge mal Druck des im normalen Blute zur Verfügung stehenden O_2 für das Bewusstsein, bei normalem Blut pH

Zur Wirkung der Alkalose auf das Gehirn

In der physiologischen Literatur ist allgemein bekannt, dass normale untrainierte Menschen in der Höhe oder im Unterdruck oder auch bei O_2-Mangelatmung im Tal in grossen Räumen oder bei ständiger Absorption der ausgeatmeten CO_2 dann an die Grenze des Bewusstseins gelangen, wenn die O_2-Spannung des arteriellen Blutes auf 30 mm (=50% O_2-Blutsättigung) abgesunken ist. Nach Christensen u Krogh fallen junge Leute bei Fliegereignungsprüfungen also dann zusammen wenn die O_2-Spannung ihrer Lungen unter 35 mmHg abfällt. Dies entspricht ungefähr 5% des Atmosphärendruckes (ref. bei Y Henderson, 1941)

Hierbei ist die alveoläre CO_2 von ca 40 mm Spannung in der Regel auf Werte von ungefähr 20 mm gesunken als Folge der durch O_2-Mangel bedingten Atemsteigerung. Es resultiert dadurch eine messbare Verschiebung des Blut-pH nach der alkalischen Seite in der Höhe oder im Unterdruck Bei Pendelatmung im Tal in grösserem Raum mit fortlaufender CO_2-Absorption konnte an der Grenze des eben noch Erträglichen ein 9 4%iger O_2-Gehalt in der restierenden Luft durch Haldane festgestellt werden (S 223) Im grossen Gegensatz hierzu konnte eine Pendelatmung mit nur 6 Litern Luft mit teilweiser CO_2-Absorption der O_2 bis auf 4 6% hinab er braucht werden. Dies erhellt deutlich den durch die Bohrkurven (O_2-Bindung von verschiedenen CO_2-haltigen Luftgemischen) nicht voll zu erklärenden Effekt der Alkalose worauf auch Haldane auf S 188 hingewiesen hat.

In ähnlicher Weise konnte auch Gellhorn zeigen, dass Versuchspersonen welche in einer Luft von 7% O_2 (=ca 50 mm O_2-Spannung) obligat in kurzer Zeit bewusstlos werden diesen O_2-Mangel ohne weiteres ertragen können, wenn der Einatmungsluft 3% CO_2 zugefügt wurden. Diese Angaben konnten wir durch Untersuchungen unseres Schülers Leuppi anhand von 23 Versuchspersonen in einer Inauguraldissertation bestätigen

Dieser so grosse Unterschied lässt sich meiner Meinung nach nicht durch die Bohrkurven erklären, denn der besseren Ausnützung des O_2 im saureren Blute bis auf tiefere Spannungswerte hinab steht die durch die CO_2-Zugabe gehemmte O_2-Sättigung entgegen Beide Faktoren halten sich also beim 7%igen O_2-Atmungsversuch ungefähr die Waage, worauf auch Opitz aufmerksam gemacht hat.

Es soll daher näher auf den reinen alkalotisch bedingten Hyperventilationsschaden aufmerksam gemacht werden, der für das weitere Verständnis äusserst wichtig ist.

Ich habe meinen Schüler Neuenschwander 80 Versuche an gesunden, 22–30-jährigen Versuchspersonen ausführen lassen Bei Zugabe von 4–6% CO_2 konnten Atmungsgemische mit nur 4–5% O_2 bei einem Barometerstand von 710 mmHg (entsprechend einer O_2-Spannung von 28,5–35,5 mmHg in der Einatmungsluft) dank der nun möglichen, grossen Atmung ohn CO_2-Verlust, also ohne Alkalose 3–4 Minuten lang störungsfrei ertragen werden. Hernach waren die Atmungssäcke jeweils leer Gleichzeitig ergaben Selenzellenwerte am histaminisierten Ohrläppchen nach der Methode von Matthes bestimmt einen arteriellen Blut-O_2-Wert von 20% entsprechend einer Spannung von 15 mm O_2

Eine willkürlich gleich gross gehaltene Atmung bei gleicher O_2-Spannung aber ohne CO_2 führte dagegen durch die respiratorische Alkalose schon in 70 Sekunden zur Störungsschwelle mit Schriftfehlern und baldiger Bewusstlosigkeit.

Ein weiterer Hinweis für alkalotisch bedingte Störung der Gehirnfunktion wird durch die Hyperventilationstetanie geliefert.

Durch die alkalotische pH Verschiebung geschieht die für die Tetanie nötige Teil-Entionisierung des Calziums, worauf ich 1951 hingewiesen habe. Infolge dieser alkalotisch bedingten Verschiebung des Blut-pH am Abend des ersten Höhenaufenthaltes (z.B Jungfraujoch 3450 m) treten nun auch bei Ruhelage nach zahlreichen eigenen Versuchen bei gesunden jedoch nicht sonderlich an Höhenaufenthalte gewöhnten Versuchspersonen z T heftige Kopfschmerzen auf Diese sind alkalotisch bedingt und werden nach eigenen Versuchen durch Atmung von 3–5% CO_2 innerhalb von 20 Sek zum vorübergehenden Verschwinden gebracht, nicht jedoch durch Atmung von reinem O_2 *sofern das Atemvolumen gleich gross gehalten ird e bei de Luft tmung*

Nun sind aber Kopfschmerzen auch bei der CO-Vergiftung zur Genüge bekannt. Jedoch gelang es uns im Gegensatz zum Höhenkopfschmerz nie durch CO_2-Atmung (3–4%) diesen CO-Kopfschmerz zu beheben Er ist also nicht alkalotisch sondern acidotisch-hypoxisch bedingt und beruht auf periarteriellem Ödem der dilatierten Schädel- und Gehirngefässe mit langer Nachdauer (Wolff 1948 Lenggenhager 196_)

Im Gegensatz zur CO-Atmung können nun (besonders bei jüngeren Menschen) infolge der ungewohnten Höhenalkalose selbst i Ruhelage richtige vegetati e Gewitter entstehen mit Brechen und Schweissausbrüchen. Dagegen fehlen bei der CO-Vergiftung nach zahlreichen eigenen Versuchen mit jüngeren liegenden Versuchspersonen diese egetativen Gewitter

Dagegen ist *am Ende* der CO-Atmung nach eigenen Untersuchungen das Atemvolumen *im Liegen* nicht erhöht, sondern kann sogar am Schluss der 30-minütigen CO-Atmung abnehmen, s Abb 4 und Abb 1

Abb. 14. Selbst in Atm. O_2 treten die für die Hyperventilation typischen Schriftstörungen auf. Sie sind also hier rein alkalotisch bedingt.

Eine Erklärung hierfür wird im Abschnitt „Warum ist das Rubeatemvolumen bei CO-Atmung anfänglich leicht ergrössert" gegeben.

Dass die akute hyperventilationsbedingte Alkalose (neben erschwerter O_2-Abgabe aus dem *Blute) noch einen Schaden* sui generis darstellt, habe ich in früheren Arbeiten beschrieben (1947 u. 1951). So zeigt B Abb. 14 schwerste alkalotische Schr torung einer 22jährigen Versuchsperson forci er H perventilation in unserer Überdru kamm *m O bei 2 Atmosphären O_2-l* H tte der plasmatisch gelöste C an genügt zur Erhaltung des Bew tsei beim Ausfall aller Erythrocyten (z. B d Belegung, was ja Haldane schon im Mau ment bewiesen hat). Die beschriebenen Sch ngen mussten also *alkalotisch* und nicht hypoxisch bedingt gewesen sein.

Übrigens finden sich solche grotesken Schriftstörungen immer nur bei O_2-Mangelatmungsversuchen nie jedoch bei rein hypoxischen CO-Zuständen (s. Abb. 1)

In einer Arbeit habe ich (1951) auch nachgewiesen, dass psychische und tetanische Erscheinungen durch alkalotisch bedingte Teilentionisierungen des Calciums auftreten können. Dies ging besonders drastisch bei einem 25-jährigen Patienten hervor welcher auf Grund eines nicht behandelten appendicitischen Abscesses durch *Spontanperforation* 2 hohe Dünndarmfisteln aufwies. Während zweier Tage stellten sich deliröse Schreianfälle ein, die erst verschwanden, als der starke Blut-CA Mangel erkannt und korrigiert worden war Es erfolgte hernach schlussendlich völlige Heilung.

Auch dem Psychiater sind akute deliröse Zustände bei Hypocalcaemie bekannt. Schliesslich sei auch daran erinnert, dass langsam i. v. verabfolgte ⅛ n NaOH beim narkotisierten Tier (Kaninchen, Meerschweinchen) zum Tode führt, bevor es zur Bildung von Methaemoglobin und Haemolyse *schädlicher* Ausmasse gekommen ist (eigene Versuche).

Banche verabreichte i. v. Alkalien bei Patienten mit Magen- und Duodenalgeschwüren und sah bei stärkerer Dosierung schockartige Zustände mit Zittern, Schwitzen und Kopfschmerzen.

Die Vorstellung, dass mit zunehmender respiratorischer Alkalose die cerebrale zellkatalytische Funktion bei Untrainierten im Schnellversuch leidet, geht wohl auch aus der Hyperventilationsohnmacht hervor

Schon Haldane erwähnt die Hyperventilationsohnmacht, schreibt aber dass deren Erklärung aus den Bohrkurven allein nicht zu geben sei Er erklärt sie in Anlehnung an Henderson Tierversuche als zur Hauptsache durch den Blutdrucksturz bedingt. Diese Hyperventilationsversuche Henderson s waren aber an tracheotomierten Tieren maschinell ganz ausserordentlich stark forciert worden, wobei sogar die Exspirationen unter raschestem Sog geschehen mussten, und sie dauerten zudem sehr lange, sodass schon die hierdurch bedingte Abkühlung der Tiere allein zum Erlöschen der spontanen Atemtätigkeit und zu den starken Blutdruckstürzen führen konnte.

An der folgenden Tabelle I unserer Eigenversuche ist aber ersichtlich, dass bei 4 gesunden

Tabelle 1 *Fehlen von Blutdrucksturz bei starker Hyperventilation im Liegen trotz schon positiver Tetanie*

	Blutdruck vor Versuch (systolisch) (mmHg)	Blutdruck bei Auftreten Kribbeln od. Tetanie bei starker Hyperventil (mmHg)	Dauer der Hyperventilation (Min.)
1 24 j ♂	125	120	11
2 26 j ♀	110	100	6
3 22 j ♀	105	110	4 ½
4 24 j ♂	128	125	5
Durchschnitte	114.5	113 7	

Versuchspersonen bei kräftigster Hyperventilation in Lieglage bis zum Auftreten von starken Kribbelgefühlen und von Tetanie praktisch noch keine Blutdrucksenkung erfolgt, obwohl in diesem Stadium schon stärkste Schriftstörungen sich geltend machen.

In der Literatur finden sich Angaben über Blutdruckabfall mit Hypozirkulation, durch respiratorische Alkalose bedingt, z. B bei Henderson Deshalb wiederholten wir an 3 weiteren, gesunden Versuchspersonen den Versuch mit der Modifikation dass in Liegelage die maximale Atemfrequenz und Tiefe ausgeführt wurden (1 Atemzug/Sekunde) bis zum Auftreten von Koma mit spontanem Atemstopp und z. T klonischen Zuckungen was je nach der Versuchsperson in 1 ½–3 ½ Minuten der Fall war 1 keinem dieser 3 Kurzversuche kam es zu einem Druckabfall im Gegenteil trat bei mir eine Blutdruckerhöhung von 120 mm (vor dem Versuch) auf 200 mmHg nach 3 Minuten und von 190 mm nach 3 ½ Minuten beim Eintritt der Bewusstlosigkeit, auf Nach Erwachen aus dem Koma wurde ich ungehalten weil ich glaubte man hätte die geplante Blutentnahme aus einer Cubitalvene zur Bestimmung des venösen Blut-pH beim Koma-Eintritt vergessen bis man mir den kleinen Wund erhand an der Punktionsstelle zeigte Mein Alter betrug z Zt dieses Versuches 58 Jahre das meiner Mitarbeiter 76 und 77 Jahre In allen 3 Fällen bestand nicht die geringste Schwierigkeit für die Venenpunktion da die Venen durchaus normal gefüllt blieben was für normale periphere Zirkulation spricht Zur weiteren Prüfung der in der Literatur noch strittigen Frage der *peripheren* Blutzirkulation während starker respiratorischer Alkalose gingen wir folgendermassen vor

) körpereigene Erythrocyten wurden diffus in ein Ohrläppchen injiziert. Nach 2 Stunden wurde durch eine Selenzelle der Gewebs -O_2 in diesem Ohrläppchen bestimmt zunächst bei Ruheatmung, dann bei O_2-Atmung ferner bei stärkster Hyperventilation in gewöhnlicher Luft bei O_2-Mangelatmung und nach völligem Unterbruch der Blutzirkulation.

Die Abb 15 zeigt dass während stärkster Hyperventilation der Gewebs-O_2 dieser Erythrocyten ebenso erhöht war wie bei Atmung von reinem O_2, während er in der darauffolgenden Apnoephase stark und nach Unterbrechung der Blutzirkulation des Ohrläppchens durch schmale Klemme (oder durch Leerpressen der Gefässe des Ohrläppchens durch angepresste Glasscheibchen) maximal abfiel näheres siehe in der Originalarbeit von Lenggenhager (1947)

Umgekehrt ergaben sich deutliche Unterschiede dieser extravasalen Erythrocyten–O_2-Werte wenn anstelle gewöhnlicher Lufthyperventilation gegen nur 10% O_2-haltiges Luftgemisch in gleicher Weise hyperventiliert wurde Jetzt nahm der Gewebs-O_2 der extravasalen Erythrocyten von ungefähr 77 auf ungefähr 70 Skalenteile ab (s Abb 15*a*)

Nun darf man aber diesem Versuch nicht zu viel Bedeutung zumessen, da CO_2-verarmtes Hb sich leichter mit O_2 aufsättigen lässt. Um diesem Einwand zu begegnen, haben wir noch eine andere Versuchsanordnung gewählt.

b) Beweisender erwiesen indessen auch die Hyperventilationsversuche wenn in durch Iontophorese histaminisierte Arm- und Beinhaut Indigoleukoblauquaddeln aus der gleichen Vorratsspritze stammend gesetzt wurden und die Zeiten bis zu deren Blaufärbungen verglichen wurden.

Während stärkster Hyperventilation waren die Färbungszeiten kürzer als bei Ruheatmung, denn

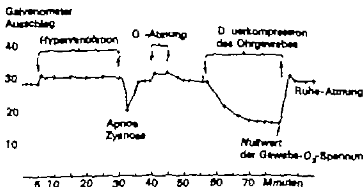


Abb 15 O_2-Gehalt im histaminisierten Ohrläppchen-*Gewebe* vor und während starker Hyperventilation bei O_2-Atmung und nach Zirkulationsunterbruch.

lich verlangert jedoch bei Hyperventilation von 10% und 8.4% O_2. (Näheres siehe in der Originalarbeit Lenggenhager 1947.)

Setzten wir diese stark forcierten Hyperventilationsversuche weiter fort (wobei bei einigen Versuchspersonen ständige Ermahnungen nötig waren), so trat wiederum kurze Bewusstlosigkeit auf

Dagegen durfte die O_2-*Bestimmung* am histaminisierten Ohrläppchen nach Matthes nicht zur Lösung der Frage des Gewebs-O_2-Gehaltes bei Hyperventilation benützt werden, *da hierbei nur der O_2-Gehalt* der *intravasale* Erythrocyten gemessen wird und nicht der Gewebe-O_2-Gehalt.

Durch diese Versuche ist der Beweis geliefert, ... während stärkster hyperventilatorischer Alkalose die O_2-Versorgung des *Haut*gewebes *durchaus eine gute* ist, was wegen der erhöhten Pulsfrequenz, dem praktisch normalen Blutdruck und der stärkst gesteigerten Lungenbelüftung durchaus begreiflich erscheint.

Hingegen gibt dieser Versuch keine Antwort darüber, ob während dieser Hyperventilation die Hautgewebe normal oder vermindert atmen, indem schliesslich der O_2 im Hautgewebe auch ungenutzt sich vorfinden könnte (was jedoch mit Rücksicht auf das weniger *spezifizierte Haut*gewebe nicht wahrscheinlich ist).

Zur Erklärung der Alkaloseohnmacht müsste man jedoch die in der Physiologie vertretene Ansicht einer mit zunehmender Alkalose einhergehenden *cerebralen arteriellen Vasokonstriktion* berücksichtigen (Kety u. Schmidt, 1946), während der periphere Blutdruck konstant bleibt (Kontos u. Mitarb.). Allerdings kommt diese Erklärung für die beschriebene, nach nur 8–10 tiefen Atemzügen *auftretende Ohnmacht* bei *leichter* CO-*Vergiftung* sicher nicht in Betracht.

Im Gegensatz zu diesen Arbeiten ist anderseits nach Singh u. Mitarbeitern die cerebrale Durchblutung bei der Höhenkrankheit (also bei O_2-Armut) gesteigert. Anderseits geschieht unter Hyperventilation nach Kety u. Schmidt (1948) eine Erhöhung des cerebralen Gefässwiderstandes. Aber trotz der herabgesetzten Hirndurchblutung blieb die O_2-*Aufnahme* in Gehirn normal, was das Vorhandensein einer wesentlich *allgemeinen* Hypoxieschädigung des Gehirns durch Hyperventilation widerlegt, für welche die Abnahme des O_2-Verbrauches sonst ein Leitsymptom darstellt (*Kety u. Schmidt*, 1946).

Auch erwiesen sich längere mässige Hyperventilationsphasen an *narkotisierten* Menschen deren Bewusstseinszentrum ohnehin ist durchaus nicht als schädlich. Sie Anästhesisten direkt gefordert, licheren Acidose mit Sch (Tschirren, Geddes u. Gr u. Parkers, etc).

Übrigens tritt nach eine Überatmungstetan schen nicht mehr in ... sich doch meiner M

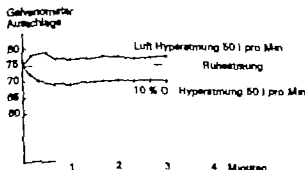


Abb 15 zeigt, dass schon in 10 ... O_2 die Erythrozyten des *Ohrgewebes bei H*... pera... ... von 50 *l/min* deutlich geringeren O_2-Gehal... ... bei gleichgroßer Luftatmung.

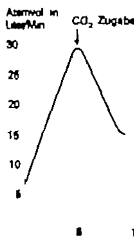


Abb 16 Stärkste Hyperventilation beim Einatmen eines Luftgemisches von 7% O_2 und 93% N_2. Bei Zugabe von 3% CO_2 Stabilisierung der Atmung auf ein Niveau von 15 Litern pro Minute Durchschnitt von 7 Versuchspersonen.

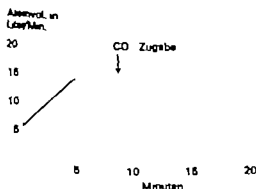


Abb 16b Mittelgrosse Atmung in einem Luftgemisch von 7% O_2 und 93% N_2, bis auf 15 Liter pro Minute steigend. Bei Zugabe von 3% CO_2 bleibt die Atmung konstant. Durchschnitt von 9 Versuchspersonen.

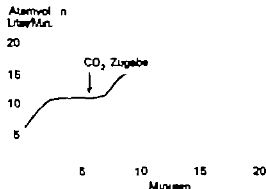


Abb 16 Bei einem Luftgemisch von 7% O_2 und 93% N sehen wir nur einen kleinen Anstieg bis auf 12 Liter pro Minute Es folgt aber mit 3% CO_2 doch noch eine Steigerung der Atmung bis auf 15 Liter pro Minute Durchschnitt von 7 Versuchspersonen.

dampfender Effekt dieser Hyperventilation *für das Bewusstsein* darin, dass solche nur mit Lachgas und Relaxantien narkotisierte, überventilierte Patienten unserer Chirurgischen Klinik in eine tiefere Narkose zu bringen sind als ohne Hyperventilation, sodass keine zusätzliche Narkotika gebraucht werden müssen (Tschirren)

Die Frage einer möglichen cerebralen Hypocirkulation im alkalotischen Gehirn des wachen Menschen musste also experimentell noch genauer erbracht, resp bestätigt werden. Sollte sich eine solche bestätigen, so wäre dies eine zusätzliche Erklärung zum alkalotischen Gehirnschaden mit der durch stärkste Hyperventilation bedingten Ohnmacht.

Durch Atmung O_2-armer Luft können nach Untersuchungen meines Schülers Leuppi bei ungefähr 1/3 der ‿3 untersuchten Studenten sehr starke Hyperventilationen ausgelöst werden. Dies wurde schon durch Haldane auf S 184 seines Buches ‚Respiration beschrieben Jedoch haben wir zuerst in einem Eigenversuch dann in einer Inauguraldissertation von Leuppi gefunden, dass bei solchen Versuchspersonen eine Zugabe on 3% CO_2 bei gleichbleibendem O_2-Mangel der Einatmungsluft das Atemminutenvolumen sich paradoxerweise stark verkleinert (s Abb. 16). Dies kann nicht durch den Bohreffekt erklärt werden da ein anderer Teil der Versuchspersonen bei Atmung gleicher Gasgemische weder die sehr starke Hyperatmung aufweisen noch auf CO_2-Zugabe die nur mittelstark vergrösserte Atmung steigern. Erst die restlichen Versuchspersonen zeigten in O_2-armer Luft keine wesentliche Steigerung des Atemvolumens. Sie wurden einfach progressiv cyanotisch bis zum Präcoma. Zugabe on CO_2 in diesem Stadium führte zu grosser Atmung und damit zur ‚Erholung (s Abb 16*e*)

Diese Beobachtung zeigt den zunächst erregenden Effekt des alkalotischen Blutes auf das Atemzentrum *bei einem Teil der normalen Versuchspersonen* Auf eine in *mittleren* Höhen (3500 m) beim Menschen existierende Herabsetzung der Reizschwelle (also im Sinne einer besseren Ansprechbarkeit aller Reflexe) haben u a. Grandjean

Tabelle 2 *Tödliche Wirkung von i v verabreichter NaOH im Kaninchenversuch welche nicht durch die resultierende Hämolyse erklärt wird*

Menge n/NaOH in ccm	i Inj Dauer	Hamolyse	Resultat
3 ½	4	Stark	†
3,0	2 ½	Stark	†
2 ½	4	Stark	†
2,0	1	Stark	†
2,0	1	Stark	†
1,5	1 ½	Mittelstark	†
1,5	1	Mittelstark	†
1,0	30'	Mittelstark	Überlebt
1 0	45'	Mittelstark	Überlebt
1,5	45	Mittelstark	Überlebt
2 0	10'	Mittelstark	Überlebt
½ n/NaOH			
8 cc	4	Stark	†
6 cc	2'	Stark	†
9 cc	12'	Stark	Überlebt

ferner auch Fleisch und v Muralt 1944 hingewiesen. Dieser Erregungszustand kann bei weiterer Hyperatmung oder O_2-Mangel jedoch ins Lähmungsstadium ubergehen

Als Ausdruck eines cerebralen Alkaloseschadens fand ich ferner folgendes an mir und an solchen unden Versuchspersonen die gleich mir bei Mangelatmung ein stark gesteigertes Ruhe-olumen aufweisen, wenn man in Liegelage 3 tiefe Atemzüge Stickstoff atmet und den Atem bei der letzten N_2-Inspiration anhält, so kann man kurz vor Einsetzen des imperativen Atemreizes Zeichen vorubergehender Hypoxie beobachten entweder Flimmern oder Verdüsterung des Gesichtsfeldes oder klonische Zuckungen der Kopf oder Gesichtsmuskulatur Alle diese Symptome verschwinden wieder kurz or Einsetzen des gebieterischen Atemreizes weil der Alkaloseschaden trotz der progressi en Hypoxie durch die CO_2-Anreicherung kompensiert wird

Ein direkter Alkaloseschaden geht nach eigenen Versuchen auch daraus hervor dass leicht or narkotisierte Kaninchen bei ntra enoser Injektion von ca 1,5–3 ccm n/Natronlauge terben. Die hierdurch auftretende Teilhamolyse ist zu schwach um den Exitus zu erklaren da Injektionen von hämolysiertem Blut mit der gleichen Schlusskonzentration ohne schädliche Wirkung erlaufen I Tab 2 sind die genaueren Verhaltnisse dargestellt

Nun kann sich aber der Organismus m t der Zeit auch an die sonst ungewohnte Alkalose gewohne

In diese Richtung weisen Untersuchungen von Cameron der die Höhenanpassung nicht eigentlich in der Wiederherstellung eines normalen Gewebs-O_2-Druckes sieht sondern vielmehr in der Fähigkeit des Körpers den Gasaustausch trotz erniedrigten O_2-Druckes aufrecht zu erhalten

Auf die Bedeutung der ,3-Phosphoglyzerate fur die O_2-Sättigungskurve des Blutes wird später kurz eingegangen

Umgekehrt f ndet sich auch eine vorübergehende Störung vor bei Menschen, die immer in der Höhe lebten (Anden) und dann ins Tal abstiegen

Mit der Zeit tritt eine Gewöhnung auch bei (jüngeren) Tieren an die Hohenhypoxie auf welche sich selbst nach längerem Talaufenthalt nicht verliert ohne dass Verzar (1960) dafur eine Erklarung geben konnte Dies scheint fur eine zellkatalytische Verbesserung der O_2-Verwertung im höhenakklimatisierten Gehirn zu sprechen denn dies kann anhand der Hb–O_2-Bindungskurven nicht erklart werden So konnten wir durch den höhengewohnten Alpinisten André Roch (der an einer Himalaya-Expedition teilgenommen hatte) im Forschungsinstitut Jungfraujoch ein Atmungsgemisch von 7% O bei einem Barometerdruck *von nur 495 mmHg atmen lassen was einem O_2*-Partialdruck von nur 33 mmHg in der Einatmungsluft entspricht! Dieses Gemisch konnte bis zum Ende des 200 Liter fassenden Atmungssackes geatmet werden ohne dass es zu merkbaren Storungen kam wohl aber wurden grosse langsame und tiefe Atemzüge von je 3 Litern geatmet. Diese Versuchsperson war also an die Alkalose so gewöhnt dass deren cerebrale Schäden sich nicht mehr (oder erst bei stärkerem Grade) einstellten.

Im Gegensatz hierzu wurden alle 5 sich zu diesem Versuch hingebenden Versuchspersonen unserer Gruppe innerhalb von *1–1 / M* n *bewusst*los

Schon Barcroft und auch Hurtado (1928) fanden, dass langjährige Höhenbewohner keineswegs eine hohere O_2-Sättigung des Blutes aufweisen und betonten dass gute Höhenverträglichkeit nicht an hohe O_2-Sättigung des Blutes gebunden sei

Desgleichen berichtete Campbell 1927 anhand von wochenlangen Tierversuchen mit Atmung O_2-armer Luft, dass die Gewöhnung nicht in Erreichung normaler Gewebs-O_2-Drucke sondern ielmehr auf der Fahigkeit beruhte trotz nied-

riger O_2-Spannungen in den Geweben den Gasaustausch aufrecht zu erhalten

Im gleichen Sinne sprechen auch die Untersuchungen von Lahiri u. Mitarbeitern (1967) und Lahiri (1968), ferner alle „blue babies" mit angeborenem Herzfehler und der beschriebene asphyktische Asthmaanfall mit dem stündigen höchst cyanotischen Komazustand und nachfolgender völliger Restitution.

Nach allen diesen Besprechungen über die respiratorische Alkalose scheint also das Gehirn das hauptleidtragende Organ zu sein. Es ist auch das weitaus empfindlichste Organ gegen O_2-Mangel.

Ein Gegengleich zu einer durch akute Alkalose bedingten funktionellen Hirnschädigung findet sich nun auch in einem durch Schädigung der Gehirngefässe bedingten Hirnschaden vor, welcher zu Störungen der metabolischen Oxydationsprozesse durch die Lactacidose bedingt ist (Ingvar u. Lassen). Erhebliche derartige Veränderungen können innerhalb von Minuten eintreten. Die saure pH-Verschiebung führt dabei zu einer massiven und langanhaltenden Vasodilatation, die als „Vasoparalyse" bezeichnet wird. Diese kann nach Ingvar u. Lassen länger andauern als die Periode der Anoxie/Hypoxie selbst, auch wenn die Durchströmung wieder hergestellt ist, sodass der Durchfluss die Bedürfnisse des Gewebsstoffwechsels längere Zeit überschreitet, welchen Zustand man als Luxusperfusion bezeichnet.

Es ist diese stark acidotisch bedingte Schädigung der Hirnzellen eine Art Gegengleich zu akut alkalotischen Hirnschädigung. Ein acidotisches Gehirn arbeitet ebenfalls nur noch bei hohem O_2-Angebot.

Nun interessierte uns die Frage, ob nicht mit neuesten Geräten auch eine klinisch zu erwartende Herabsetzung der O_2-Spannung für die Endaufsättigung des normalen, aber an CO_2 verarmten menschlichen Blutes festzustellen wäre. Die bisher üblichen O_2-Bestimmungen im van Slyke-Apparat scheinen für die letzten Sättigungsgrade hierfür nicht geeignet wegen der zu kleinen für die Analyse zu verwendenden Blutmengen.

Wir bedienten uns daher wiederum des vorgängig beschriebenen spektrometrischen CO-Oxymeters, welches auf 1% genau die O_2- und CO-Sättigung des Blutes bei 37°C angibt (s. Abschnitt „Zur Kritik an der Haldane'schen Auffassung").

Versuchsbedingungen für die O_2-Sättigungskurven bei verschiedenen CO_2-Spannungen von 40, 20, 10 und 0 mm Spannung CO

1. Verwendet wurde menschliches, heparinisiertes Frischblut, mit N_2 reduziert (früher bereits beschrieben).

2. Gemische von je 150 l mit verschiedenen O_2- und CO_2-Spannungen werden in einem 200 l fassenden Gummisack stets frisch hergestellt und mit der auf S. 15 beschriebenen Formel errechnet. Die Ergänzung der CO_2- und O_2-Mengen auf 150 l geschieht jeweils mit reinem O_2-freiem N_2.

3. In Abb. 6 ist das genaue Versuchsschema gezeigt. 4 cc Blut werden durch Hahn A in das mit dem jeweiligen Gasgemisch vorgefüllte System eingefüllt. 10 Minuten lang wird abwechslungsweise das Blut kräftig in dem Kolben geschüttelt und nach je ca. 30" wieder neues Gasgemisch mittels Wasserstrahlpumpe durchgesogen. Für die Blutproben-Entnahme gelten die gleichen, früher angegebenen Kriterien.

Die Untersuchungen meines Dissertanten Spycher mit diesem Gerät ergaben nun die aufschlussreiche Tatsache, dass mit zunehmender CO_2-Verarmung die O_2-Bindungskurven des menschlichen Blutes steiler verlaufen *unter progressiver Linksverschiebung auch ihrer Endsättigung*. Dies heisst also, dass die O_2-Spannung im alkalotischen Erythrocyten (bei gleichem O_2-Druck der Sättigungsluft) kleiner ist als im normalen CO_2-haltigen Blut, weil der O_2 leichter aufgenommen wird. Die Abb. 17 zeigt diese Ergebnisse meines Schülers Spycher. In Abb. 17 sind diese Verhältnisse bildlich dargestellt. Es scheint aber nicht ausgeschlossen, dass bei stärkerer Hyperventilation die O_2-Verwertungsschwelle für das Bewusstsein sogar noch auf 40 mm Spannung heraufgetrieben wird, was allerdings in der Abb. 17a nicht berücksichtigt wurde, wohl aber *in* der Abb. 18.

Diese progressive Herabsetzung der O_2-Spannung in einem zunehmend hyperventilatorischen Blute, zusammen mit der alkalotischen Erschwerung der cerebralen O_2-Verwertung (mindestens im Bewusstseinszentrum) dürfte also die Hauptrolle spielen für die Erklärung der Hyperventilationsohnmacht. (Dieser Alkaloseschaden wird durch allfällige cerebrale Hypozirkulation noch verstärkt.)

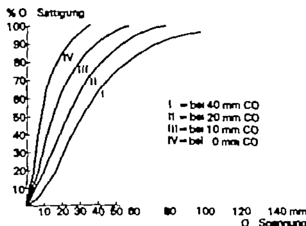


Abb 17 Neue O_2-Sättigungskurven des menschlichen Blutes bei abnehmendem CO_2-Gehalt, bestimmt mit dem modernen „CO-Oxymeter" bei 38°C

Der Begriff eines lokalisierten, cerebralen Bewusstseinszentrums ist nicht neu. Breslauer hatte schon vor 40 Jahren an in Lokalanästhesie operierten Hunden nachgewiesen dass im basalen Hirnstammgebiet auf kleiner Fläche durch sachteres Beklopfen Bewusstlosigkeit auftreten kann, nicht aber von anderen Hirnteilen aus.

1945 habe ich anlässlich einer in Lokalanästhesie ausgeführten Kleinhirn-Tumor-Entfernung auch beim Menschen ein solches Bewusstseinszentrum in tragischer Weise bestätigen können. Nach Entfernung des ganzen rechten Kleinhirns blutete es hartnäckig aus einer kleinen Stelle der vorderen Medulla oblongata, sodass schliesslich ein milder und ganz kurzer Elektrokoagulationsstoss auf diese Stelle ausgeführt wurde Die indifferente grossflächige Elektrode lag unter dem

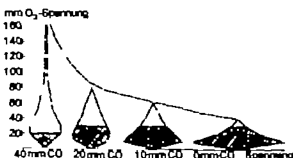


Abb 17 Bildliche Darstellung (der Abb 17) mit der erleichterten O_2-Aufnahme in progressiv CO_2-ärmeren Erythrocyten. Daraus geben der zunehmende O_2-Spannungsverlust und die abnehmende O_2-Menge für das Bewusstsein hervor

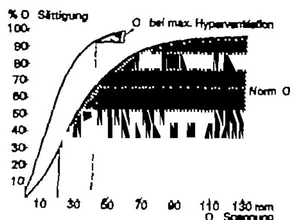


Abb 18 Die obere, kleinschraffierte Fläche zeigt die für das Bewusstsein unterschwellige O_2-Menge bei stärkster Hyperventilation durch alkalotische Verschiebung der lokal-cerebralen O_2-Verwertungsschwelle von 20 auf 40 mm Spannung (punktierte senkrechte Linie). Die untere schraffierte Fläche entspricht dem O_2-Angebot bei normalem Blut.

Gefäss dieser 32jährigen, erblindeten Patientin Im Moment der kurzen Koagulation erlosch schlagartig das Bewusstsein dieser vorher immer ansprechbaren Patientin für ca 1 Minute Nach Erholung musste leider eine zweite in der hinteren Pons-Gegend gelegene hartnäckige Blutsickerstelle endlich auch durch kurzen Elektrokoagulationsstoss gestillt werden. Jetzt erloschen leider definitiv Bewusstsein Atmung und Herzaktion. *Die beiden Stellen sind in Autopsiepräparat der Abb 19 bezeichnet.* Alle proximal dieser Stellen liegenden Gehirnteile waren vom Stromstoss verschont, *damit ist ein lokalisiertes Bewusstseinszentrum auch beim Menschen erwiesen*

Die Tatsache dass auch schwere Gehirnverletzungen durchaus nicht immer zu Bewusstlosigkeit führen müssen haben wir öfters beobachtet. So brachte ein Patient, welchem ein Lastwagenrad langsam über den rechten Stirnpol gefahren war bei Bewusstsein die aus der offenen Schädelfraktur in den Hut ausgepressten Stirnhirnmassen mit ins Spital und zeigte sie uns!

Auch haben wir Hirndurchschüsse durch kleinkalibrige Revolverschüsse erlebt ohne Bewusstseinsverluste wenn die Kugeln z.B fronto-frontal verliefen, gelegentlich sogar mit inneren Reflexionen.

Auch quere Hinterhauptabtrennung durch Propellerverletzung führte nach Spatz nicht zu Bewusstseinsverlust. Es bedarf zu letzterem eben der

Druckwellenfortpflanzung in dem hinteren Hirnstamm, wo das Bewusstseinszentrum liegt.

Zusammenfassung über die Alkaloseschäden

Nach dem Besprochenen setzt sich also ein Hyperventilations Alkaloseschaden aus folgendem zusammen:

1 Erschwerte O_2-Angabe aus dem alkalotischen Blute wegen stärkerer Bindung an das Hb selbst in den obersten Sättigungsgraden.

2. Hierdurch schädliche Herabsetzung der für das Bewusstsein so wichtigen O_2-Spannung.

3 Gleichzeitige Heraufsetzung der für ein alkalotisches Bewusstseinszentrum eben noch genügenden minimalen O_2-Spannung.

4 Alkalotisch bedingter spezifischer Gehirnschaden unabhängig vom O_2-Mangel (deshalb auch in der O_2-Überdruckkammer auslösbar).

5 Teilentionisierung des Calciums durch die Hyperventilationsalkalose was u. a. zu cerebral bedingter Tetanie führt

6 Allfällige alkalotisch bedingte cerebrale Hypozirkulation.

Alle diese Faktoren summieren sich zum Bilde des schweren Hyperventilationsschadens.

Kombination on Alkalose und CO

Kommt nun zu den besprochenen Wirkungen der Alkalose mit der Erschwerung der cerebralen O_2-Verwertung und der gleichzeitig alkalotisch bedingten Herabsetzung der Blut–O_2-Spannung noch eine Teilbelegung des Blutes durch CO so gesellen sich also nochmals 2 schädliche Faktoren dazu. die durch CO bedingte Verringerung der O_2-Menge und damit deren Spannung.

Unsere anfängliche Vorstellung über den Grund der raschen Bewusstlosigkeit durch Hyperventilation beim Vorliegen einer CO-Teilbelegung des Blutes war die Annahme, es könnte ich der Bohreffekt in einem solchen CO-Blute tarker auswirken. Doch wird dies durch die Untersuchungen von Haldane nicht bestätigt:

Die Blut–CO-Sättigung wird bei progressiver CO_2-Verarmung im gleichen Verhältnis orverlegt wie im normalen Blut (S 165 Haldane)

Im Gegensatz zu Laborversuchen mit O_2-Bindungsversuchen eines CO-teilvorgesättigten Blutes geschieht also bei der menschlichen CO-Vergiftung das Umgekehrte- das Lungenblut das zunächst nahezu O_2-gesättigt ist kommt progressiv mit einschleichendem CO in Berührung Letzteres wird sich also om oberen Ende der



Abb 19 1 Stelle der Medulla oblongata, von wo aus durch schwächste Electrocoagulation Atmung u. Bewusstsein schlagartig ausgelöscht wurden. 2 Stelle in hinterer Pons, wo nach schwächster Electrocoagulation Koma u. Herzstillstand ausgelöst wurden. 3 Schnittfläche des r Hirnschenkels. 4 Vierhügelgegend. 5 linke Kleinhirn-Hemisphaere. 6 breit offener 4. Ventrikel. 7 Schnittfläche des Halsmarkes.

Hb–O_2-Bindungskurve her progressiv binden lassen, weil dort die geringste O_2-Affinität herrscht, und damit der CO-Bindung zuerst kein Hindernis entgegen gebracht wird.

Neue Erklärung der Hyperventilationsohnmacht bei CO-Vergift ng

Nach dieser Besprechung des Kombinationsschadens von CO-Wirkung und Alkalose können nun die Verhältnisse beim Hyperventilationskoma endlich befriedigend erklärt werden.

Betrachten wir Blut einer nicht trainierten Versuchsperson, welches zu 25% durch CO nachbelegt wird

In Ruhe kann dieser verringerte O_2 bei normalem pH bis hinunter zu 20 mm O_2-Spannung dem Gehirn zur Verfügung stehen. Kommt aber eine CO_2-Verarmung infolge kurzer maximaler Hyperventilation dazu so wird wegen dieser Alkalose die für das Bewusstsei nötige minimale O_2-Spannung auf mindestens 30 mmHg hinauf gedrückt wie wir das von der Höhenkrankheit wissen, wo wegen des gesteigerten Atemvolumens es schon bei einer 30 mm O_2-Spannung zur Bewusstlosigkeit kommt. Dazu kommt nun also die hyperventilationsbedingte Linksverschiebung der O_2-Bindungskurve welche nicht nur zur Herabsetzung der O_2-Spannung, sondern gleichzeitig noch zur alkalotisch bedingten Verringerung der für das Bewusstsein nötigen O_2-Menge führt.

I Abb 11 sind die noch erheblichen O_2-Produkte aus Menge mal Spannung in einem 25%

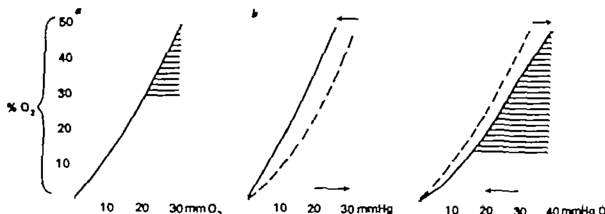


Abb 20 In *a b* und handelt es sich um Blut, dessen oberste 50% durch CO beladen sind. ( ) Genügend O_2 für den Bewusstseinszustand im Liegen (*b*) Bei kurzer Hyperventilation ist kein O_2 für das *Bewusstsein mehr vorhanden Koma. CO_2=20 mm bedingt* eine Linksverlagerung der O_2-Bindungskurve mit gleichzeitiger Rechtsverschiebung (also Erschwerung) der cerebralen O_2-Verwertung. (*c*) *Bei CO_2-Atmung* (80 mm CO_2) starke Vergrösserung des erfugbaren O_2 infolge Rechtsverschiebung der O_2-Bindungskurve. Die unterbrochenen Linien stellen die normale O_2-Bindung *in normalen Blut* dar dessen obere 50% durch CO-Nachsättigung belegt sind bei einer normalen CO_2-Spannung von 40 mm.

CO-Blut für das Bewusstsein dargestellt im Gegensatz zum unterschwelligen O_2-Produkt bei kurzer Hyperventilation, was zum Koma führt Die Abb 12 entspricht den normalen Verhältnissen.

In Abb 13 ist ersichtlich, warum ein Mensch 50%iger CO-Blutsättigung (Feld 1) besser ht als bei 25%iger CO-Sättigung mit Hyper latlon Würde jedoch ein Mensch mit 50%iger CO-Blutsättigung nur kurz hyperventilieren so ergäbe sich überhaupt kein für das Bewusstsein zur Verfügung stehender O_2-Vorrat mehr

Die folgende Art der Darstellung eignet sich noch besser zur Wiedergabe der O_2-Spannungsverminderung eines normalen Lungenblutes durch nachträglichen 25%igen CO-Beschlag: In einem 25% durch CO befallenen Lungenblut hört die O_2-Bindungskurve abrupt bei 75% auf in einem 50% CO-Blut bei 50% Von diesen O_2-End sättigungspunkten steigen die CO-Kurven nahezu senkrecht an, da schon unter 1 mm CO-Spannung eine *totale Blutsättigung* erreicht wird Dies wird durch die Abb 20 dargestellt.

Nun beginnt also die O_2-Nachsättigung eines zu 50% durch CO vorgefüllten Blutes schon bei 1 mm O_2-Spannung. Der O_2-Nachsättigung sind also die sonst für eine 50%ige Blutsättigung nötigen 30 mm Spannung durch die 50%ige CO-Vorsättigung abgenommen worden. Damit ist auch der O_2-Endsättigungsdruck erniedrigt worden Umgekehrt pfropft sich auch das CO auf ein zu 50% durch O_2 orgesättigtes Blut schon unterhalb 1 mm CO-Spannung völlig auf Es dringt dabei also zunächst wiederum an der untersten, noch nicht besetzten Hb-Stelle ein und erfüllt also von der Mitte der Sättigungskurve an aufwärts den Rest der Erythrocyten. Dies muss man aus den Haldane schen Kurven die sich bei zunehmenden CO-Drucken auf ein O_2-vorgesättigtes Blut ergeben (S 166 Abb 9) schliessen.

Nochmals zu Gegenüberstellung von CO und Höhenatmung

Haldane macht mit Recht auf eine gewisse Ähnlichkeit zwischen Bergkrankheit und CO-Vergiftung aufmerksam (S 240) obwohl er bei der CO-Vergiftung eine normale O_2-Spannung im Blute annimmt. Nach neuer Auffassung ist dieser Vergleich noch zutreffender da in *beiden* Fallen die O_2-Spannung herabgesetzt ist. Nur findet sich die Höhenatmung wegen des vergrösserten Ruheatemvolumens mit einer *respiratorischen Alkalose vergesellschaftet, die* CO-Vergiftung jedoch mit einer Acidose.

Die Alkalose ist nach Haldane nicht nur am ersten Höhentage sondern auch nach der Akklimatisierung nachgewiesen worden. (S 107 seines Buches „Respiration) Wir selbst haben eine alkalotische Blut-pH Verschiebung *in 3 Höhenexpeditionen* auf Jungfraujoch mit total 26 Versuchspersonen on 7,36 auf 7 49 während der jeweils ganzen 14tägigen Dauer bestätigen können (anhand des elektrischen Tenn-Messgerätes). *Die*

gleiche alkalotische pH Verschiebung wiesen auch 2 dauernd auf Jungfraujoch Angestellte auf

Wohl geschehen schon am 2. Höhentage durch Einströmen von Milchsäure aber auch besonders durch 2,3-Phosphoglyzeraten ins Blut und in die Erythrocyten (nach Finch und Lenfant, und auch nach Benesch et al.) nach rechts verschobene O_2-Bindungskurven der Erythrocyten. Diese Vorgänge spielen jedoch aus zeitlichen Gründen bei der akuten CO-Vergiftung noch keine Rolle

Diese Alkalose bedingt nun für Untrainierte die Erschwerung der cerebralen O_2-Verwertung für das Bewusstsein welche Funktion also nur noch unter einem höhere O_2-Angebot erhalten bleibt. Hierfür sorgt die vergrösserte Atmung. Wegen dieser Höhenalkalose wurden nun alle 5 eigenen Versuchspersonen auf Jungfraujoch (3450 m) schon bei einem Blut–CO-Gehalt von nur 15% nach ca 8 tiefsten Hyperventilationsatemzügen komatös, während es im Tale bei den gleichen Versuchspersonen erst bei 25% CO–Blutgehalt zum Bewusstseinsverlust gekommen war

Bei der Höhenatmung jedoch wirkt sich die Alkalose zunächst deshalb nicht in deletärem Masse aus weil durch das vergrösserte Atemvolumen immer eine bessere O_2-Aufsättigung des Blutes geschieht. Hierbei halten sich, wenigstens in nicht zu grossen Höhen, der schmälernde Bohreffekt einerseits und die bessere O_2-Sättigung anderseit zunächst die Waage Ganz anders bei der CO-Vergiftung. Hier besteht eine progressive hypoxische Acidose weil das Atemzentrum allmählich erlahmt, trotz der zunehmenden Anhäufung hypoxischer intermediärer Stoffwechselsäuren.

Die Acido e jedoch gestattet dem Gehirn die be sere O_2-Ausnütz g des Blute bis auf W rte soge nt 20 mm O_2-Spannu g hinab Dazu kommt nu bei s hwere acidotis hen Graden d CO-Vergiftung die Sä e-bedingte Rechtsve chiebung der O_2-B ndung as einer höheren O_2-Spannung diese Blut bei gleichbleibendem Luftdruck gleichkommt Ohne das hierdurch mehr O ins CO-haltige Blut aufg nommen werden kann ist also dennoch di O_2-Spannung ang stiegen

Dies gibt meines Erachtens eine neue Erklärung zu Haldane Feststellung, dass bei CO-vergifteten Mäusen die ersten Symtome durchaus nicht er stärkt, sondern eher gemildert werden, wenn diese Tiere menschliche Exspirationsluft bei künstlich gleichbleibendem CO-Gehalt zu atmen bekommen, obschon nun eine geringere O_2-Spannung in der Atmungsluft vorliegt. Haldane s Erklärung hierfür ist nämlich die Hebung des Blutdruckes durch bessere Herzaktion unter der CO_2-Wirkung.

Nach der neuen Erklärung jedoch erniegert die Einatmung von Exspirationsluft die O_2-Sättigung in CO-teilbelegter Lungenluft keineswegs, da infolge der CO-Beladung immer noch genügende O_2-Mengen vorliegen, um das O_2-Manco dieses CO-Blutes völlig auszugleichen. Dagegen nimmt die O_2-Spannung nun wegen der acidotischen Rechtsverschiebung der O_2-Bindungskurve zu. Dies mag aus Abb 20 hervorgehen.

Unsere CO-Versuche auf Jungfraujoch (3450 m) sind ein guter Beweis für die neue Auffassung des ersten CO-Eintrittes ins Lungenblut „von oben her" Deshalb können ja in der Höhe [3450 m] die ersten 10% der Blut–CO-Sättigung in Ruhelage vollständig symptomlos ertragen werden, da zuerst nur die ohnehin leeren obersten Partien der Hb-Sättigungskurve CO-nachgefüllt werden. Nach Haldane s Vorstellung wäre gleich von Anfang der CO-Atmung an mit einer progressiven Reduktion des verwertbaren O zu rechnen was zu einem bald auftretenden Pulsanstieg führen müsste welchen wir jedoch in der Höhe bei nur 10–12% Blut–CO-Sättigung nicht feststellen konnten

Vergleich der Gefährlichkeit on CO-Vergiftung nd Höhenatmung

Ausgehend von der Ansicht, nicht nur die physikalische, sondern auch die ‚biologische O_2-Spannung im CO-Blute sei im Tieflande eine normale vertritt Haldane die Ansicht es sei eine CO-Vergiftung nicht so gefährlich wie reiner O_2-Mangel bei Atmung O_2-armer Luft in grossen Räumen oder i der Höhe wo die tödliche Katastrophe häufiger beobachtet werde Dieser Auffassung möchte ich etwas einschränkend entgegenhalten dass auch längere hypoxische Zustände beim Atmen O_2-armer Gemische durchaus nicht immer deletär sei müssen. So konnte ich als 38-jähriger ½ Stunden in einer Unterdruckkammer für Fliegereignungsprüfungen bei einem Unterdruck entsprechend einer Meereshöhe von 9000 m ohne O_2-Atmung nur mit gelegentlicher CO_2-Zufuhr (zur Behebung der sonst periodisch auf

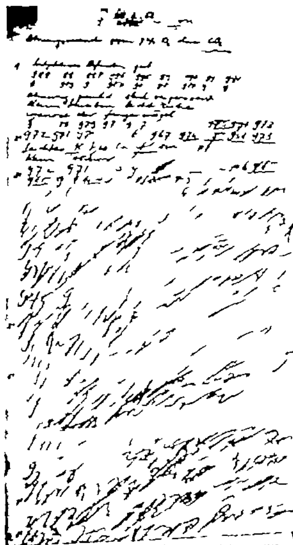


Abb 21 Über 15 Minuten anhaltende stärkste hypoxisch-alkalotische Schriftstörungen einer 23j. ♂ Versuchsperson bei Atmung von 7% O_2. (Es wurde noch ein 2. Blatt unleserlich verkritzelt!)

tretenden starken Überatmungstetanie mit Schriftstörungen) sitzend verweilen und dauernd gute Schriftproben ausführen und Rechenaufgaben lösen, wie ich das 1942 beschrieben habe.

Dr F von Tavel der die Schweizerische Militär-Unterdruckkammer für Fliegereignungsprüfungen konstruieren liess und dem ich obigen Eigenversuch verdanke erzählte mir dass er einmal in der Unterdruckkammer sitzend bewusstlos zusammensank und gemäss der Uhr in seinem Protokoll während 20 Minuten am Boden liegend im Koma verharrte um dann bei gleichbleibendem Unterdruck sich spontan wiederum so weit zu erholen dass er bewusst zur O_2-Atmung schreiten konnte

In Abb 21 sind recht langdauernde Schriftstörungen stärkster Art abgebildet, herrührend von einer 23-jährigen Versuchsperson, die in halb sitzender Lage über 19 Minuten bis zur völligen Leerung des Atmungsreservoirs ein Luftgemisch von nur 7% O_2 entsprechend einer Spannung von ca 47 mmHg (Barometerstand von 710 mmHg) atmete dauernd völlig unleserliche Zeichen kritzelte und nach dem Versuch keinerlei Störungen aufwies. (Leider sind die beiden *folgenden Seiten* dieser interessanten Schriftprobe beim Umzug unserer Klinik in ein neues Gebäude verloren gegangen)

Auch die klassisch gewordene Ballonhöhenfahrt von Sivel Tissandier und *Spinelli 1875 (Haldane* „Respiration“ S 319) welche zwar auf über 8000 *m Meereshöhe zu 2 Todesopfern führte* ergab immerhin einen die lange Bewusstlosigkeit Überlebenden mit keinerlei Nachstörungen.

Bedenkt man ferner dass die beiden anderen Ballonfahrer in sitzender Stellung im Ballonkorb bewusstlos zusammenbrachen, so ist gut vorstellbar dass wegen der resultierenden Kopfhängelage es zu einer Erstickung kommen konnt so wie auch nach längerer Narkose ein Patient in Rückenlage durch Zungenaspiration ersticken könnt wenn nicht der Unterkiefer durch eine Hilfsperson am Zurücksinken verhindert würde In der Armeeunterdruckkammer konnte ich selbst einige Male miterleben, dass junge Fliegeraspiranten im Unterdruck entsprechend einer Höhe von nur 4500-5000 m ganz plötzlich aus ihrer sitzenden Stellung heraus bewusstlos und mit Krämpfen auf den Tisch hingefallen wären hätte die O_2-atmende Begleitperson sie nicht im letzten Moment gehalten und O_2-Atmung eingeschaltet. Je nach der Körperlage eines solchen hypoxischen Menschen könnte er nun, sich selbst überlassen durch Zungenaspiration glatt ersticken.

Im gleichen Sinne spricht eine UPI -Meldung aus Madrid vom 4 6 69 wonach der 22 jährige Armando Socarras Raminez als blinder Passagier versteckt im Fahrwerkschacht einer DC 8 den fast 9stündigen Flug von Havanna nach Madrid bei einer Flughöhe von 9000 m ohne Druckkabine in bewusstlosem Zustande überlebte (Ref im „Der Bund“ Bern om 5 6.69)

Diese wenigen Beispiele sollten nur zeigen, dass unter Umständen auch starker O_2-Mangel im Zustande völliger oder teilweiser Bewusstlosigkeit während längerer Zeit ertragen werden kann. Warum es bei reinem O_2-Mangel in der Einatmungsluft erfahrungsgemäss doch rascher zu

Todesfällen kommt als bei CO-Vergiftung welche gelegentlich über Stunden eine reversible Bewusstlosigkeit mit völliger Erholung aufweisen kann beruht nach eigenen Erfahrungen z T darauf dass der CO-Vergiftete in der Regel Zeit hat, sich *schützend hinzulegen*, während der akute O_2-Mangel wegen der durch die grossere Atmung sich aufpfropfenden Alkalose ein plötzlicheres krampfartigeres Geschehen darstellt. So stürzen solche Patienten „ohne vorherige Umschau bewusstlos hin und ersticken oft wegen atmungsbehindernder Körperlage wie im zitierten Ballonunglück, also Vorkommnisse welche bei CO-Patienten wegen häufigen Fehlens des bewusstlosen Zusammenfallens seltener sind. Im Gegensatz zur O_2-Mangelatmung kommt es bei der CO-Vergiftung im Koma aber zu einem progressiv sich verkleinernden Atemvolumen mit praeterminaler stark ansteigender hypoxischer Säuerung des Blutes und der Gewebe Durch diese Säuerung wird aber gleichzeitig die O_2-Spannung (bei gleichbleibender prozentualer O_2-Sättigung) erhöht Die Acidose aber ermöglicht wiederum eine viel bessere Ausnützung des Blut-O_2 bis auf Werte von voraussichtlich 15 mm Spannung herab allerdings bei ausgelöschtem Bewusstsein, während es also bei reiner O_2-Mangelatmung im Gegenteil zu einer alkalotisch bedingten Erschwerung der O_2-Ausnützungsmoglichkeit fur das Gehirn gekommen ist, bei gleichzeitiger Linksverschiebung der O_2-Bindungskurve was einer schädlichen Potenzierung gleichkommt.

Gleichzeitig aber kennt doch jeder Kliniker auch die schweren Schäden und die vielen Todesfälle die sich bei CO-Vergifteten leider so häufig ergeben.

Umgekehrt aber konnen Menschen unter Umständen stundenlang in hypoxischem Zustande komatös verweilen (gleich wie CO-Vergiftete) *e ein normales oder gar saures Blut pH-orl gt „B bei ersticknngsahnlich Zuständen* Haldane macht auf S 204 darauf aufmerksam, und ich selbst verweise nochmals auf die 50jahrige Asthmatikerin welche postoperativ über 1 Stunde lang tiefst asthmatisch-cyanotisch und bewusstlos dalag ohne späteren Schaden zu nehmen

Damit bedarf es also nicht der Haldane schen Annahme einer normalen O_2-Spannung (mit *reduzierter O_2-Menge) für* die Erklarung der relativ langezeit erhaltenen Atemtätigkeit bei der CO-Vergiftung Die Atmung kann vielmehr selbst bei sehr niedrigen O_2-Spannungen lange erhalten bleiben sofern nur wie bei der CO-Vergiftung der Säuregrad des Blutes erhalten bleibt oder sogar zunimmt, ganz im Gegensatz zur Höhenatmung. Gleich wie unser kurzer Hyperventilationstest bei nur leichter CO-Vergiftung zum sofortigen alkalotischen Koma führt so bedingt auch eine in stark O_2-armer Luft ausgeführte Ruhe Hyperventilation rasches Coma. Der Unterschied besteht also im Wesentlichen in der stark alkalotischen pH Verschiebung bei der Höhenatmung im Gegensatz zum sauren Blute des sich selbst überlassenen Co-Patienten.

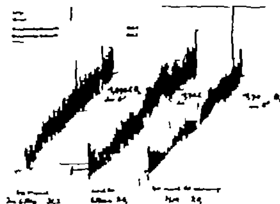


Abb 22 Linke Kurve zeigt den O_2-Verbrauch und die Atemexkursionen vor dem CO-Versuche (bei O_2-Atmung am Knipping-Apparat). *Mittlere Kurve:* Atmungskurve unmittelbar nach 30' CO-Atmung (5 ‰). *Rechte Kurve:* Atmungskurve 40' nach Beendigung der CO-Atmung. Die initiale Hypopnoe und die Verkleinerung des Atemvolumens sind Ausdruck der *getilgten O_2-Schuld (Abnahme der hypoxisch angehäuften Milchsäure*)

De Gas-Stoffwechsel bei mittelstarke CO-Verg ftu g

Aus unseren wenigen Eigenversuchen scheint der O_2-Verbrauch in Zuständen von 30–50 %iger CO-Vergiftung nur unwesentlich verringert (S *Abb 22) Wohl aber ist uns aufgefallen* dass die Atmungskurven in einem O_2 am Knipping-Apparat gemesse ei deutlich kleineres Totalvolumen ergeben Diese Abnahme des Ruheatemvolumens *nach* abgeschlossener CO-Atmung verdeutlicht sich sogar mit Latenz.

In Abb 22 ist ein Beispiel einer solchen Atemregistrierung im Liegen sofort und 40 Minuten

Pulsfrequenz
80
70
60

0 10 20 30 40 60 Zeitdauer der CO Atmung I Min

Abb 23 Regelmässige Anstiege der Ruhepulse mit zunehmender CO-Atmungszeit in Liegelage. Durchschnitt on 9 Versuchspersonen

nach Beendigung der halbstündigen Atmung von 50‰ CO in Luft gegeben Aus unseren Versuchen ergibt sich das unerwartete Ergebnis dass der O_2-Verbrauch trotz des um nahezu 1/3 kleiner gewordenen Totalatemvolumens praktisch gleich gross geblieben ist Die Versuchspersonen *mussten* vor dem Versuch ! Stunden lang vorliegen um ihr Ruheatemvolumen als Ausgangswert vor dem CO-Versuch zu liefern

Der Grundumsatz bei 30 %iger CO-Vergiftung·

geprüft wurden männliche Versuchspersonen

	vor Versu h	*nach CO-Atm ng*
a) 30 j	+3 %	+20 %
b) *47 j*	+7 %	+12 %

Versuchsperson a) atmete *33* ein *5‰ CO-*Luftgemisch Versuchsperson b) atmete 48' ein 5‰ CO-Luftgemisch

Die Versuche wurden jeweils im direkten Anschluss an die CO-Atmung am kleinen Knipping-Apparat ausgeführt.

Bei ruhig liegenden Menschen scheint sich demnach schon nach leichteren CO-Vergiftungen von nur 30 % Blut–CO-Gehalt mit der Zeit eine O_2-Schuld *einzustellen die dann durch* eine reine O_2-Atmung rasch getilgt wird So kommt es zu einer erheblichen Verkleinerung des Atemminutenvolumens wie dies aus der Abb 22 hervorgeht, wo das Atem olumen um 30 % abnahm

Damit ist wieder ein Hinweis dafür gegeben, dass das erste Organ welches unter der CO-Einwirkung in Mitleidenschaft gezogen wird das Gehirn sein muss und nicht primär andere innere Organe ansonst der O_2-Verbrauch schon in den leichteren Stadien der CO-Vergiftung zu sinken begänne

Zu unseren CO-Versuchen bis zur 30 %igen Hb-Sättigung ist noch z sagen, dass ausser einer gewissen Dösigkeit leichtem Kopfschmerz im Liegen (mit der beschriebenen Kopfrötung und dem höheren Ruhepuls) beim gewöhnlichen Arbeiten eine massig starke Dyspnoe auffiel Diese machte sich bei mir besonders geltend als ich ersuchsweise mit dem Fahrrad kurz nach einem Eigenversuch nach Hause fuhr Die kleine Anhöhe die dabei zu ersteigen war musste ich diesmal wegen Atemnot erstmals zu Fuss *erklimmen. Dafür hatte ich* nachher die Genugtuung, dass die Erholungszeit (gegenuber früheren und auch späteren CO-Versuchen ohne anschliessende stärkere körperliche Betätigung) verkürzt wurde

Warum ist das Ruheatem olumen bei CO-Atmung anfa glich leicht vergrö sert

Unsere Versuchspersonen bei welchen wir das Atemvolumen während der Atmung eines *5 %*igen CO-Luftgemisches massen zeigten in den ersten 20 Minuten einen leichten Anstieg des Atemminutenvolumens der gegen das Ende der gewöhnlich 30–35 Minuten dauernden CO-Atmung wiederum einer Verkleinerung bis unter die Werte der Norm Platz machte ein Beispiel s in Abb 4

Nimmt man die Erklärung Haldane s an wonach die O_2-Spannung im CO-Blute eine normale sei weshalb selbst bewusstlose CO-Patienten kein vergrossertes Atemvolumen aufweisen so ist diese ungefähr 10 %ige Steigerung des Ruheatemvolumens unserer ausgeruhten Versuchspersonen während der ersten Hälfte der CO-Atmung nicht erklärlich Es sieht vielmehr so aus also ob die verminderte O_2-Spannung im ZNS im Wachzustande sich auszuwirken beginnt, sodass eine Stressreaktion mit Aussendung zentraler Impulse ausgelöst wird Hierfur sprechen einmal das subjektive H *tzegefühl mit dem gelegentlich geröteten* Gesicht das leichte Zittern der Finger und die leichte Pulsfrequenzerhöhung In Abb 23 und die schon bald mit zunehmender CO-Atmungszeit auftretenden Pulsfrequenzerhöhungen dargestellt als Durchschnittswerte von 9 gesunden liegenden Versuchspersonen. Sie beginnen schon in den ersten 10 Minuten der CO-Atmung

Zorn hatte 1972 an *Ratten Kaninchen* und Katzen den interessanten Nachweis erbracht dass die Abnahme des O_2-Partialdruckes im *Gehirn*gewebe bei CO-exponierten T eren nahezu reziprok linear dem Anstieg des CO-Sättigung im Blut verläuft. Diese Senkung des O_2-Partialdruckes

Abb. 24 zeigt den nach CO-Atmung bald eintretenden Atemstopp in Exspirationsstellung bei narkotisierten Kaninchen und Meerschweinchen mit langer Apnoe, dann mit vereinzelten, immer rascher auftretenden Schnappatemzügen (mittlere Reihe), bei sich allmählich wieder normalisierender Atmung (untere Reihe).

im Gewebe erfolgt schon durch niedrige CO-Konzentrationen des Blutes.

Für eine solche Auffassung sprechen indirekt auch die Versuche meines Schülers Lehmann an narkotisierten Meerschweinchen und Kaninchen, bei welchen *unter dem Einfluss der Narkose* diese Stressreaktion trotz Atmung eines 20%igen CO-Luftgemisches ausbleibt. Es kommt vielmehr *bei diesen tracheotomierten Tieren einfach zu* einem plötzlichen Sistieren der Atmung (s. Abb. 24).

Gleich verliefen seine Versuche, wenn solchen narkotisierten und tracheotomierten Tieren i. v. Natriumnitrit injiziert wurde: wiederum setzte die Atmung ohne vorherige Vergrösserung plötzlich aus (s. Abb. 25). Das Natriumnitrit hatte, wie postmortale Blutuntersuchungen zeigten, die Bildung von Methämoglobin bedingt, das für den O_2-Transport völlig wegfiel. Es konnte gleich wie das Blutplasma nur noch rein physikalisch geringste Mengen O_2, aber unter normalem Druck, aufnehmen und deshalb die intraarteriellen Chemorezeptoren nicht im Sinne eines O_2-Mangels erregen. Dagegen erhielt nun das Atemzentrum ungenügende O_2-Mengen und versagte ohne Aussendung von Warnreaktionen.

Comroe und Schmidt fanden schon 1938, dass ein völlig CO-beladenes, aber dann kurz mit O_2 durchperltes Blut den tierischen Carotis-Sinus nicht mehr *erregte zum Aussenden atemsteigernder Impulse ans Atemzentrum*, im Gegensatz zu einem

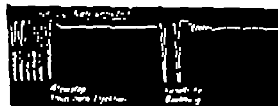


Abb. 25 zeigt den plötzlichen Atemstopp in Exspirationsstellung aus normaler Atmung heraus bei i. v. Natriumnitrit-Injektion beim Kaninchen (narkotisiert).

Tabelle 3

Durchschnittswerte Atemvolumina Liter/Minut

Versuchspersonen	In Luft (l)	Luft+CO_2	Nach CO-Atmung Luft+CO_2
1 47 J. ♂	4,8	mit 3% Co_2 9,5	mit 3% CO_2 9,5
2. 33 J. ♂	5,8	mit 5% Co_2 20,0	mit 5% CO_2 17,0
3 27 J ♂	4,6	mit 6% Co_2 15 4	mit 6% CO_2 17 0
4 27 J ♂	5,5	mit 7% Co_2 17,8	mit 7% CO_2 17,0
5 36 J ♂	5 0	mit 7% Co_2 17,5	mit 7% CO_2 32 0
Durchschnittswerte	5,0	16 0 l	18,5 l

CO-durchspülten Blute weil im letzteren kein plasmatischer O_2 mehr war und die Erregung des Carotis-Sinus wiederum auftrat

Diese Verhältnisse eines kaum oder nicht vergrösserten Atemvolumens bei *Einatmung* geringer CO-Mengen gelten nur für den ruhenden Körper oder den narkotisierten. Ein sich bewegender Mensch verspürt nach CO-Einatmung verstärkte Atemnot und weist damit eine Dyspnoe Atmung auf da die Muskelmilchsäure für Erregung des Atemzentrums sorgt. Sie verschwindet bei der CO-Vergiftung sowie auch in der Höhe unter Einfluss des O_2-Mangels langsamer als üblich weshalb das Atemvolumen durch eine gleiche Anstrengung sowohl während der CO-Vergiftung als auch in der Höhe viel stärker zunimmt und einer längeren Erholungszeit bedarf als im Normalfalle

Zur verminderten Ansprechbarkeit des Atemzentrums gegenüber CO bei CO-Vergiftung

Eigene Versuche mit 5 verschiedenen liegenden Versuchspersonen zeigten dass bei nur 30%igen Blut-CO-Befall die Anprechbarkeit des Atemzentrums gegenüber dem CO_2-Reiz noch nicht abgenommen hat, fast alle zeigten bei Einatmung eines 3–7%igen CO_2-Luftgemisches eine prompt auftretende und prozentual ca gleichbleibende Steigerung des Atemvolumens wie dies vor dem CO-Versuch der Fall war Es war also das Atemzentrum bei diesen leichten Graden der CO-Vergiftung noch durchaus in der Lage auf CO_2-Reiz normal zu reagieren (s Tabelle 3)

Auch das Blut-pH war in diesem Stadium noch unverändert (5 Versuchspersonen) Dagegen konnte gelegentlich sogar ein stärkerer atemsteigernder CO_2-Effekt im Stadium dieser CO-Vergiftung festgestellt werden

In Tabelle 2 sind 5 Beispiele zusammengestellt, worunter in 2 Fällen ein stärkerer atmungssteigernder CO_2-Effekt im CO-haltigen Organismus vorliegt Da O_2-Spannung und Menge im *CO-Haemoglobin* herabgesetzt sind muss sich nach meiner seit 1942 vertretenen Ansicht die CO_2 in leichterem noch nicht längere Zeit bestehendem CO-Blut eher stärker auswirken bei hohem O_2-Druck dagegen geringer

Im Gegensatz zu diesen leichteren CO-Vergiftungen bewirkt aber ein starker CO-Befall im späteren Stadium mit Koma eine geringe Ansprechbarkeit des Atemzentrums gegenüber dem CO_2-Reiz. Deshalb wird dann das Atemvolumen kleiner unter starkem Anstieg der Blut und Gewebssäuerung. Diese Tatsache ist bekannt und findet sich u a. auch bei Zorn Die Schuld an dieser Acidose trägt der O_2-Mangel unter welchem das Atemzentrum zu versagen beginnt Erst stärkste Säurereize vermögen in diesem Stadium noch vereinzelte grosse ‚Seufzer' Atemstösse hervorzubringen. Werden Meerschweinchen welche in CO-Luft bewusstlos hinfallen und keinerlei Atembewegungen mehr zeigen, in diesem Stadium in eine kleine O_2-Überdruckkammer eingeschleust, so erholt sich die Atemtätigkeit wiederum über den Weg einer initialen, grossen und frequenten Atmung. Selbst wenn durch akute CO-Vergiftung bei Zufuhr von 20% CO die Meerschweinchen innerhalb einer Minute bewusstlos und völlig atemgelähmt wurden konnten sie sich zu unserem Erstaunen spontan erholen, wenn sie sofort in reine Luft gebracht wurden Alsdann sieht man, dass nach ungefähr 20–30 Sekunden ein erster tiefer Atemzug erfolgt, nach weiteren ca 15 Sekunden ein zweiter Dann folgen sich diese tiefen ‚Schnappatemzüge immer häufiger und nach einigen Minuten ist die Atmung einigermassen nor

mal und regelmässig geworden. Die Tiere erholen sich spontan!

Abb 24 zeigt eine unserer vielen Atmungskurven von auf obige Weise CO- ergifteten Meerschweinchen mit spontaner Erholung.

Das Blut-pH von 8 solchen Tieren ging im Momente des längsten Atemstopps von 7,54 auf 7,22 herunter! So stark ist die Lähmung des Atemzentrums in diesem Stadium! Durch diese starke Säuerung kann aber der O_2 bis auf allergeringste Spuren im CO-Körper erbraucht werden, im Gegensatz zur Alkalose welche im Gegenteil die zentrale Verwertbarkeit des O_2 verschlechtert. Letzteres ist wohl der Grund für das unterschiedliche Verhalten von Patienten im O_2-Mangel und bei CO-Vergiftung.

Unsere Meerschweinchenversuche stehen im Gegensatz zu Angaben von Haldane der fand, dass Mause die in einem Überdruck on 2 Atm. O_2 und 1 Atm. CO sich normal verhielten beim Ausschleusen jedoch akut an CO-Vergiftung starben. Untersuchungen meines Schulers Tromp konnten dies zwar fur Mäuse oll bestätigen, nicht dagegen für die grösseren Meerschweinchen welche sich spontan nach schwerster CO-Vergiftung (in Kurzversuchen) in Frischluft erholen konnten. Es muss dies mit dem höheren Grundumsatz kleiner Lebewesen zusammenhängen, wo sich ein O_2-Mangel starker auswirken muss. Diese spontane Erholungsfähigkeit nach akuter CO-Vergiftung bis zum Koma wurde 1969 nun auch durch Schittek an Ratten bestätigt.

Die At mankalt möglichkeit während der CO-Vergiftu g

Interessehalber bestimmten wir bei unseren CO-Versuchen das maximale Atemanhaltevermögen in der Vorstellung dass dieses erheblich herabgesetzt würde. Wir waren anfänglich recht erstaunt dass dies nicht der Fall war So betrug beispielsweise mein maximales Atemanhaltevermögen or dem Versuch 85 n Liegelage nach ,2 Minuten der Atmung eines 7%igen ( ) CO-Luftgemisches im mer noch 83 ( ) Durchschnittswerte aus je 3 Versuchen

Die Erklarung für diese zunächst für uns unerwartete Tatsache wird folgende sein wohl sind die Erythrocyten nur je bi zur Hälfte mit O_2 gefüllt (die oberst Hälfte jeweils durch CO) sie werden aber nach Teilentleerung in den Kapillaren durch die Spannung des O_2 der Lungenluft rasch immer wieder bis zum möglichen Ende der O_2-Bindungskurve nachgefüllt. Dies aber geschieht im Gegensatz zur Norm viel leichter weil weniger O_2-Spannung hierzu nötig ist. Da aber gleiche O_2-Spannung und Menge in der Lungenluft vorliegen wie in der Norm, erhält sich auch die ursprüngliche Atemanhaltemöglichkeit beim mittelstark CO-vergifteten Menschen.

Zur Genese des CO-Kopfschmerzes

Allen unseren 12 Mitarbeitern ist immer wieder die sehr bekannte Tatsache des lästigen Kopfschmerzes im unmittelbaren Anschluss an die CO-Vergiftung aufgefallen der oft über einige Stunden anhält. Obwohl er sich subjektiv gleich äussert wie der Höhenkopfschmerz, scheint mir doch diese Genese eine andere denn während der Höhenkopfschmerz nach eigenen Untersuchungen (196.) ein *alkalotischer* Kopfschmerz ist der durch Einatmen von 4–5% CO_2 innerhalb von 20 Sekunden vorübergehend ganz behoben werden kann ist dies beim CO-Kopfschmerz durchaus nicht der Fall. Man darf wohl für letzteren mit Wolff das konsekutive Ödem der Schädel (und Gehirn) Arterien-Adventia-Schichten für diese lang anhaltenden Kopfschmerzen erantwortlich machen welches auf Grund der starken arteriellen Kopfgefässerweiterungen entsteht und welch letztere auch schuld sind am subjektiven Hitzegefühl und der sichtbaren Gesichtsrötung. Die Genese dieses Kopfschmerzes ist also wesensgleich mit den durch i Histamininjektion ausgelösten Kopfschmerzen, sie werden ebenfalls (nach eigenen Untersuchungen) durch O_2-Atmung nicht beeinflusst

Diese deutliche Kopfrötung mit dem bald einsetzenden Kopfschmerz war den früheren Hausfrauen anlässlich ihrer Bügelarbeiten mit den holzkohlegeheizten Bügeleisen allgemein bekannt und beruhte auf leichter CO-Vergiftung Es ist dieser Kopfschmerz übrigens das erste subjektive unangenehme CO-Frühsymtom Nach eigenen Untersuchungen wird dieser CO-Kopfschmerz (wie übrigen auch der Histaminkopfschmerz) durch O_2-Atmung recht lange Zeit n cht beeinflus t (dies steht m Gegensatz zur Angabe on Zorn der ein sehr rasches Nachlass n des Kopfschmerzes durch O_2-Atmung angibt) Daneben bemerkten wir nseren CO-Versuchen als weiteres Frühsymtom zusammen mit der Kopfrötung

ein leichtes Zittern der Finger und einen leichten Pulsanstieg

CO-Wirkung in der Höhe

Die öfters vertretene Meinung eine leichte CO-Vergiftung müsste sich in der Höhe viel verderblicher auswirken als in der Talluft (s z.B Jordi, aber auch bei Haldane auf S 239) hat sich uns zunächst nicht bestätigt Wir haben 6 Mitglieder einer eigenen Jungfraujochexpedition im Tal (560 Meter über Meer) und am ersten Tage auf Jungfraujoch (3450 Meter) CO in ausgeruhter Liegelage atmen lassen im Tal 5‰, auf Jungfraujoch 4,5‰ (beide Werte unreduziert) um durch ev Blockierung der Carbanhydrase den sonst häufigen, alkalotischen Kopfschmerz zu vermeiden oder zu unterbrechen. Zwar ist uns letzteres nicht gelungen dagegen aber hat keiner der Teilnehmer irgendeine Änderung in seinem Befinden während oder nach der 15minütigen CO-Atmung empfunden, wahrscheinlich weil keine Arbeitsleistung erheischt und Liegelage innegehalten wurde Es ist dies nach unserer neuen Vorstellung über die Wirkungsweise der CO-Vergiftung verständlich da das CO ja nur die oberste durch O in der Höhe ohnehin nicht oder nur mit Mühe belegte Partie der Hb–O_2-Sättigungskurve besetzt. Dies aber bedeutet für den Höhenorganismus keinen wesentlichen Abbruch.

Douglas u. Haldane atmeten ein O_2-armes Luftgemisch entsprechend einer Höhe von 5000 m. In diesem Zustande konnte eine Blut-CO-Sättigung von 23% ohne merkliche Störung ertragen werden (auf S 279 des Buches von Haldane). Diese Angabe steht im Gegensatz zu dem Postulat auf S 239 von Haldane, welches oben erwähnt wurde.

Dagegen wurden wiederum, wie im Tal unsere CO-Versuchspersonen nach ungefähr 6–8 tiefen und raschen Hyperventilationsatemzügen vorüber gehend bewusstlos wie dies früher beschrieben und erklärt wurde.

In ähnlicher Weise hatten Haldane und Smith (1897) zu ihrem grössten Erstaunen gefunden dass CO-teilvergiftete Mäuse O_2-Mangel nicht schlechter ertrugen als normale Kontrolltiere Dies gab ja den Anlass zur systematischen Untersuchung über die O_2-Bindungskurven eines teilweise durch CO-belegten Blutes

Wohl aber müsste es dann in grösseren Höhen zum Unglück kommen wenn es durch stärkeren CO-Einbruch ins Blut nicht nur zur Belegung der O_2-freien oberen Hb-Partien sondern noch zu einer zusätzlichen massiveren Verdrängung des O_2 aus der verbleibenden Hälfte der Bindungskurve des Hb käme Hier müsste die höhenbedingte Steigerung des Atemvolumens zur besprochenen alkalotischen Rechtsverschiebung (=Hemmung) der *O_2-Verwertungskurve im Gehirn* und damit zur rascheren Bewusstlosigkeit führen.

Für eine solche Auffassung sprechen übrigens unsere besprochenen Selbstversuche auf Jungfraujoch (3450 m) Daselbst konnten schon bei einer Blut-CO-Sättigung von nur ca 15% die gleichen Komasymptome durch 6-8 tiefste Hyperventilationatemzüge ausgelöst werden, weil in der Höhe schon eine leichte Alkalose vorliegt Im Tale bedurfte es einer 25%igen CO-Sättigung für die gleichen Hyperventilationssymptome

In ähnlicher Weise wurde ebenfalls unsere 40-jährige weibliche Versuchsperson im Zustande einer 30%igen CO-Blutsättigung im Tal nach 4 ½-minütiger Atmung eines 10%igen O_2-Gemisches (mit N) bewusstlos weil durch die Steigerung des Atemvolumens von 7 lt pro Minute *auf 11 lt es zu* der verderblichen Alkalose gekommen war welche sich im CO-Blut so drastisch auswirkt. So wurde die Schwelle des Bewusstseins unterschritten In der nachfolgenden Luftatmung geschah baldige spontane Erholung im Liegen. Im vorhergehenden Kontrollversuch mit Atmung eines 10%igen O_2-Gemisches (vor der CO-Vergiftung) trat keine Bewusstlosigkeit auf wohl aber die erhebliche Steigerung des Atemvolumens. Dadurch aber konnte die Blut–O_2-Sättigung deutlich zunehmen im grossen Gegensatz zum CO-Versuch Bei letzterem brachte die Hyperatmung nicht nur keinen Gewinn, sondern im Gegenteil nur einen alkalotisch bedingten O_2-Verlust für das Gehirn.

Werden durch die menschlich CO-Vergiftung auch Gewebs Katalysatore gehemmt?

Diese in der Literatur gelegentlich vertretene Ansicht (z.B Meldrum u Roughton (1934) ferner Warburg (1926) und andere) stützt sich allerdings nur auf ausserordentlich starke CO-Konzentration bei Gewebsexplantaten oder Hefezellen mit Gasgemischen die weit über 50% CO aufweisen was bei der menschlichen CO-Vergiftung praktisch nie vorkommt (vergleiche hierzu die bereits beschriebenen eigenen Versuche meines Schülers Schläpfer)

Warburg hatte 1926 zudem nachgewiesen, dass die Affinität des CO für Cytochromoxydase niedrig ist und nur ca 1/10 derjenigen on O_2 ent spricht.

Ein starkes Argument gegen d Ansicht von menschlicher G ebs-Katalysoren-Vergiftung durch CO erblicke ich in der Arbeit on Martland u Martland o 2 Fälle tödlicher CO-Vergiftung schwangerer F auen seziert u den ohne dass im foetalen Blute CO-Spu en nachgewiesen erden konnten

Offensichtlich genügte selbst diese hohe Blut-CO-Sättigung noch nicht, um die Placentar schranke welche für die foetale O_2-Versorgung gut passierbar ist zu überschreiten

Es ist dies erständlich wenn man sich an die ungeheuer geringen CO-Drucke erinnert, die zur 60–70 %igen (gewöhnlich tödlichen) Sättigung des Blutes führen. Sie liegen unter einem Millimeter Hg

Die gleiche Ansicht, dass das CO unter so niedrigen Drucken ans Haemoglobin gebunden sei, da es bei der CO-Vergiftung des Menschen prak tisch nicht ins Gewebe hinein diffundieren könne wird auch durch Elbinger ertreten. Letzteres könnte erst dann eintreten wenn der Partialdruck des CO n der Einatmungsluft sehr hoch wäre was aber wegen fast augenblicklicher totaler Blockie rung der Erythrocyten ebenso rasch zum sofor tigen Tode führen könnt wie die Na N int njektion mit sofortiger Blockierung der Erythro cyten m Tierversuch Auch im letzteren Falle werden in der kurzen Zeit keinerlei Zellfermente gehemmt weshalb es in der O_2-Überdruck kammer nicht zur sonst raschen Schädigung sol cher Tiere kommt

Übrigens ist die CO-Affinität für Cytochromoxydase nur etwa 3/10 von jener für O_2. Die Verhältnisse für Cytochrom seien noch unklar (nach Kreuzer)

Desgleichen prechen Haldane Versuche mit Mäusen, welche sich n einer Überdruckkammer von 3 Atm n einem Gemisch on 1/3 CO nd 2/3 O_2 durchaus normal erhielten und erst beim Ausschleusen einer bewus tlos zusammen brachen und bald tarben gegen die Ansicht einer Hemmung von Gewebskatalysatoren durch CO Diese Versuche konnte mein Schüler T omp in einer Dissertation a oll bestätigen

Haldane hatt ferner an Hb-f eien Insekten (Küchenschaben) die Ungiftigke t des CO selbst in 80 %iger Konzentration ( ) mit 20 % O_2 be wiesen, indem diese T ere 14 Tage lang in dieser Atmosphäre ohne jegliche Störungen leben konn ten (auf S 3 seines Buches Respiration, 1935).

Eigene Versuche mit Meerschweinchen, welche in einer 10 %igen CO-Luft in kurzer Zeit komatös und öllig atemgelähmt hinfielen, verliefen eben falls äusserst interessant. sofort in einen O_2-Überdruck on 3 Atm erbracht erholten sich diese Tiere in kurzer Zeit und konnten nach unge fähr 10 Minuten wiederum ausgeschleust werden ohne sichtbaren Schaden aufzuweisen. Damit musste der Grossteil des Blut-CO im O_2-Übe druck w ederum abgeraucht sein Blutuntersu chungen ergaben nur noch CO-Mengen von 15–20 %

Primäre oder ekundäre Herzschädigung du ch CO

Haldane (auf S 237 seines Buches „Respiration") schloss aus dem raschen Kollabieren CO-ver gifteter Menschen bei Gehversuchen und seinem Eigenversuch mit der Neigung zum Hinfallen und dem Übelkeitsgefühl auf eine primäre CO-be dingte Herzschädigung, welche er für diese Zu stände anschuldigte Dafü schien auch zu sprechen dass die Atmung selbst in bewusstlosen Zuständen noch lange erhalten bleiben konnte Die bei überlebenden CO-Patienten häufig beobach teten Herzdilatationen schienen ihm eine Bestäti gung für diese Ansicht

W selbst sind durch unsere zahlreichen Tier und Eigenversuche wenigstens für CO-Kurzver suche zu anderer Ansicht gelangt.

Leitet man z. B du ch einen dichten mit Abzug ersehenen Meerschweinchenkäfig ein 20 %iges CO–Luftgemisch so werden die Tiere in kurzer Zeit bewusstlos Hierbei hört die Atemtätigkeit sofort auf bei noch längere Zeit schlagendem Herzen Versetzt man nun diese bewusstlosen, atemgelähmten Tiere in eine kleine und daher rasch auf Überdruck zu br ngende Überdruck kammer mit 2–3 Atm einem O_2 so erholen sich sämtliche dieser bewus tlosen und atemgelähmten Tiere innerhalb weniger Minuten Sie beginnen zwar m t einer grossen und frequenten Atmung welche sich zur Tilgung der O_2-Schuld (mit der Anhäuf ng hypoxischer Stoffwechselsäuren) ein stellt doch schon bald normalisiert sich die Atmung und die Tiere erheben sich und gehen wiederum in normaler Weise in der Kammer

herum. Hier genügte also das passive Einpressen von O_2 in die Atemwege zur Wiederbelebung. Ein Beweis, dass hier unter CO eindeutig das Atemzentrum und das Bewusstseinszentrum primär gelitten hatten und vorerst nicht das Herz.

Auch Zorn berichtet, dass das Kreislaufversagen meist später einsetzt als das Versagen der Atmung.

Interessante Nebenbeobachtung: sogar schon 5 Minuten nach der Einschleusung konnten diese Tiere unbeschadet wiederum in gewöhnliche Luft ausgeschleust werden, ohne dass sich die zunächst zu erwartenden Störungen der Hypoxie wieder eingestellt hätten! Blutuntersuchungen dieser Tiere zeigten, dass auch bei ihnen nur noch unterschwellige CO-Mengen von höchstens 20% vorlagen. Damit ist der Beweis erbracht, dass durch den O_2-Überdruck von 2 Atm das CO in kurzer Zeit zum Grossteil das hypoxisch gesäuerte Blut wiederum verlassen hat.

Registriert man an einem narkotisierten Meerschweinchen oder Kaninchen die Atemtätigkeit in Normalluft, so erlebt man wiederum bei Zufuhr von 5–10% CO in kurzer Zeit ein völliges Sistieren der Atemtätigkeit in der CO-haltigen Luft, während das Herz weiter schlägt. Unterbricht man jetzt die CO-Atmung, so treten nach 15–30 sekundiger Pause vereinzelte „Seufzer-Atem" in immer kleiner werdenden Intervallen bis nach wenigen Minuten die Tiere wiederum eine normale Atemtätigkeit aufweisen (s. Abb. 19).

Während Haldane (auf S. 241 seines Buches „Respiration") die Ansicht vertritt, dass CO einen genügenden Grad der Anoxie bewirke zur Erzielung einer primären Herzschädigung, während das Atemzentrum noch antworte, verliefen unsere Tierkurzversuche also umgekehrt, was aus Abb. 24 deutlich hervorgeht. Offensichtlich ist das Herz mit seiner arbeitbedingten Muskelmilchsäurebildung viel besser gegen O-Mangel geschützt als das Gehirn.

Eine interessante Nebenbeobachtung schien zunächst jedoch für ein primäres Erlahmen des linken Herzens zu sprechen: akut durch CO bewusstlos hingefallene und [illegible] gelähmte Meerschweinchen konnten nicht passiv durch Thoraxkompressionen beatmet werden, indem keine Luft die Trachea verliess, auch nicht nach vorgängiger Anlage einer Tracheotomie. [illegible] diese immer wieder beobachtete Er[illegible] zuerst als Ausdruck reiner Schädigung d[illegible] Herzkammer auf, wobei der rechte Ventrikel weiterhin Blut in die Lunge pumpt und es zu einer Art kardialen Asthmas käme.

Inzwischen hat Pettinati in Hundeversuchen nachgewiesen, dass es bei 80%iger Blut-CO-Sättigung vor dem Herzkammerflimmern zum Ansteigen des Blutdruckes in der Lungenschlagader auf das 2–3-fache kommt. Hier hatte zweifellos der linke Ventrikel praeterminal zu versagen begonnen, was begreiflich erscheint. Da unsere Beobachtung jedoch nach vorgängigen Aderlässen nicht mehr gemacht werden konnte, drängte sich noch eine andere Erklärung auf: Es muss sich hierbei auch um den Effekt des starken Muskeltonusschwundes der Atemmuskulatur akut CO-vergifteter Tiere handeln, weshalb der Thorax in stärkste Exspirationsstellung zurückfällt. Dabei wirken die nun blutüberfüllten Lungen (deren Gefässquerschnitte grösser geworden sind) ganz gleich wie eine kardial gestaute Lunge. Beide können durch passive zusätzliche Thoraxkompression wegen Ventilmechanismus nicht beatmet werden, wohl aber durch tracheale Überdruckatmung.

An mir selbst habe ich nach Atmen eines 7,5%igen (!) CO-Luftgemisches in Liegelage während 25 Minuten eine Blut-CO-Sättigung von 55% erlebt. Während ich gut sprechen und auch schreiben konnte (letzteres nur etwas zitterig), kollabierte ich 2mal beim Versuch herumzugehen und verlor jeweils nach vorgängigem Übelkeitsgefühl das Bewusstsein. Hierbei sank der Blutdruck jedesmal auf nicht mehr messbare Werte, erholte *sich aber in Liegelage mit passiver Beinhochlage* in kurzer Zeit immer wieder. Dieser Versuch scheint mir dafür zu sprechen, dass es nicht primär das Herz war, das hier versagte, sondern *das Vasomotorenzentrum* und ganz allgemein die Empfindlichkeit des ZNS gegenüber O_2-Mangel, worauf früher schon hingewiesen worden ist.

Auf diese Weise kann es natürlich *auf die Dauer* schon zu einer Herzdilatation kommen, die aber dann, meiner Meinung nach, als *sekundäres* Phänomen aufzufassen ist, indem die Coronarzirkulation *infolge des Blutdrucksturzes* leidet und *sekundär* zum Schaden führen kann und EKG-Veränderungen bedingt.

Im übrigen ist wegen der Muskelmilchsäure des arbeitenden Herzens die Möglichkeit einer besseren O_2-Ausnützung bis auf erheblich tiefere O_2-Spannungen hinab gewährleistet im Gegensatz zum ZNS, welches wenigstens in Kurzversuchen

eine sehr erhebliche Abhängigkeit von den Bohr schen O_2-Sättigungskurven und der alkalotisch bedingten Erschwerung der O_2-Verwertung auf eist, was gezeigt worden ist

Auch die Tatsache dass das Myoglobin nach Millikan eine besonders hohe O_2-Bindungsfähigkeit hat, spricht für eine gewisse Resistenzmöglichkeit des Myocards gegen O_2-Mangel. So liege bei spielsweise die Halbsättigung des Myoglobins mit O_2 bei 4 mmHg, während beim Haemoglobin eine solche erst bei 30 mmHg vorliegt

Warum wird eine chronische Anaemie von 50 % Hb gut ertragen im Gegensatz zur 50 %igen CO-Blockierung der Erythrocyte

Aus der Literatur ist zur Genüge bekannt dass chronisch erfolgende Hb-Verluste (parasitär Chlorose Perniciosa usw) bis auf 50 % hinab ohne weiteres ertragen werden und sogar eine nicht zu schwere körperliche Arbeit gestatten. Selbst Patienten mit nur mehr 30 % Hb sind noch zu leichterer Arbeit fähig.

Ich konnte 2 eigene Patienten untersuchen, welche mit chronischen Anaemien in unsere Behandlung traten. Eine 4?jährige Frau mit inneren Haemorrhoiden welche bei jedem Stuhlgang bluteten, bis die Patientin ein Hb von 12 % aufwies. In diesem Stadium war sie nicht mehr gehfähig gab aber im Liegen und Sitzen normal Auskunft und zeigte klinisch keinerlei vergrösserte Ruheatmung. Durch Bluttransfusionen und Operation genas diese Patientin in kurzer Zeit.

Ein 70jähriger Magencarcinom-Patient kam mit einem Hb von nur 9 % Dieser Wert wurde wegen Zweifels meinerseits in meiner Gegenwart durch die geübte Laborschwester noch 2mal wiederholt und ergab in der Tat 1mal 9 % das 3 Mal 10 % gemessen mit dem Zeiss schen Haemoglobinometer Auch dieser Patient konnte im Liegen eine durchaus normale Anamnese geben und zeigte wiederum keine Atemnot oder Dyspnoe Atmung im Liegen. Als er sich jedoch auf den Abort begeben wollte kollabierte er erholte sich aber sofort wieder im Liegen Nach Transfusionen konnte ich auch diesen Patienten erfolgreich operieren

Im grossen Gegensatz zur chronischen Anaemie hat also wie dies Haldane und auch der beschriebene Eigenversuch gezeigt haben ein zu 50 % mit CO „Vergifteter“ an der alleräussersten Schwelle seiner körperlichen Leistung. Er erhält sich so wie unsere beiden beschriebene Patienten mit Hb-Stürzen auf ca 10 % hinab ein Gehen ist nicht mehr möglich wegen des baldigen Kollabierens

Nach der Haldane schen Erklärung ist für mich das Problem nicht gelöst, weil Haldane beim CO-Patienten zwar eine normale O_2-Spannung aber eine (wegen Teilbelegung durch CO) verminderte O_2-Menge annimmt, weshalb ein solches CO-Blut seinen O_2 nur in kleinen Mengen, jedoch normal rasch ans Gewebe abgeben könne

Da nun aber beim CO-Patienten die Zahl der Erythrocyten im Gegensatz zum anaemischen Patienten nicht vermindert ist, sollte ein 50 % ger CO-Patient doch ungefähr gleich gut dastehen wie ein Anaemischer mit 50 % Hb

Der Einwand es hätte eben der Chronisch-Anämische Zeit zur Adaptation gehabt ist aber deshalb nicht stichhaltig weil im Tierversuch eine Anämie im Akutversuch durch jeweiligen Plasmaersatz bis hinunter zu 10 % Hb ohne weiteres ertragen wird

Nach der neuen Erklärung ist die Lösung dieses Problems einfach geworden. Im zu 50 % belegten CO-Blut sind nach Abb 13 sowohl die biologische O_2-Spannung als auch die O_2-Menge ganz ausserordentlich stark herabgesetzt. Beides muss sich als Schaden potenzieren, denn O_2-Druck mal O_2-Menge entsprechen der biologischen Wirkung. Auch ist nach dieser Auffassung das so eindrückliche Bild raschester Bewusstlosigkeit durch kurzes Hyperventilieren bei CO-haltigem Blute besser erklärt

Dabei ist der rein *physikalische* O_2-Druck des CO-Blutes zwar normal hoch jedoch genügt die sehr kleine O_2-Menge in keiner Weise für eine nur minimale Erhaltung der Funktion des Atemzentrums wie durch das sofortige Aufhören der Atmung durch Infusion von Na-Nitrit im Tierversuch gezeigt worden ist

Umwelt-Temperatur und Erholungszeit bei CO-Vergiftung

In seinem Buche „Respiration“ machte Haldane auf S. 437 auf die Beobachtung von Kohlebergwerkarbeitern aufmerksam wonach sich der Zustand von schwer CO- ergifteten Menschen verschlechterte wenn sie an kalte frische Luft gebracht wurden im Gegensatz zu windgeschützten wärmeren Räumen. Eine Erklärung hierfür gibt Haldane nicht

In einer Inaug. Dissertation liess ich meinen Schüler Hunziker Untersuchungen an je 54 Tieren akut CO-vergifteten Meerschweinchen anstellen wobei 3 Gruppen bei Erholungslufttemperaturen von 4 20° und 37° untersucht wurden Da

ergab sich in Bezug auf das erste Herumgeben dieser primär druch 20% CO-Luftgemisch komatös gewordenen Tiere dass die kürzeste Erholungszeit in 20° Luft auftrat (19 45 als Durchschnittswert) die weitaus längste in 4 Luft (49 15") und eine mittlere in 37° Luft (28)

Als Erklärungsversuch hierfür sei folgende Annahme gemacht. Die komatösen CO-Tiere verhalten sich einer äusseren Abkühlung gegenüber wie hibernierte Warmblüter· Sie erleiden keine periphere Vasoconstriction und kühlen deshalb wie der narkotisierte Organismus ab Dadurch aber wird der O_2 viel leichter an das Hb gebunden und folgedessen erschwert an das Gewebe abgegeben Umgekehrt dürfte man sich vorstellen dass durch Erwärmung der komatösen CO-Tiere deren O_2-Verbrauch gesteigert wird wogegen die CO-Teilbelegung dieses Blutes eine zusätzliche O_2-Mehrversorgung nicht zulässt, denn durch die benötigte Hyperpnoe geschieht die anfangs beschriebene alkalotische Erschwerung der cerebralen O_2-Verwertbarkeit.

Zur Frage de Vielgestaltigkeit der Symptome nach CO-Vergiftung

Der Satz von Ellinger (1931) wonach sich sämt Autoren darüber einig seien dass der Um- der Nachkrankheitssymptome in keinerlei Beziehung stehe zur Schwere der akuten Vergiftung, wird allerdings durch Teleky bezweifelt. Er fand, dass der Prozentsatz der Nachkrankheiten bei den Fällen die mit langdauernder Bewusstlosigkeit begannen viel grösser ist als bei den anderen.

Unter der bisher entwickelten Ansicht, dass es bei der menschlichen CO-Vergiftung keine spezifisch toxische CO-Wirkung gibt kann die so grosse Mannigfaltigkeit der beobachteten Spätsymptome ausserordentlich vereinfacht werden bei Berücksichtigung der Tatsache dass das ZNS das primär leidtragende Organ ist Warum jedoch in einen Fall das Zuckerstoffwechselzentrum in einem andern das Vasomotorenzentrum in einem dritten das Temperaturzentrum in wieder andern mehr geistige Funktionen betroffen sind beruht meiner Ansicht nach auf der durchaus nicht immer einheitlichen Blutversorgung aller ZNS-Partien Dies wissen die Neurochirurgen seitdem man die cerebrale Angiographie macht, und dies weiss besonders auch der Pathologe der die erschiedenen Gefäss-Anomalien und -Pathologien sieht.

Nach Kreuzer treten CO-Schädigungen vor allem in Hirngebieten mit relativ schlechter Kapillarzirkulation auf z.B Globus pallidus rote Zone der Substantia nigra und an Oberflächen der Hirnrinde

So mussten wir selbst einen 41jährigen Patienten begutachten welcher nach mehrfachem Kollabieren anlässlich von Gasleitungsreparaturen schlussendlich eine tiefe Bewusstlosigkeit davontrug. Dies führte zu einer über Monate anhaltenden Störung seines Vasomotorenzentrums als alleiniges Überbleibsel der CO-Vergiftung Dieser Patient wies immer bald nach dem Aufstehen starken und progressiven Blutdrucksturz bei normaler Herztätigkeit auf und wurde von der Versicherung schlussendlich angenommen Durch langes und systematisches Trainieren konnte das Vasomotorenzentrum wiederum erzogen werden

Ich möchte also diese Vielgestaltigkeit der nervösen Ausfälle auf die hypoxische Noxe des ZNS zurückführen Damit aber vereinfacht sich wiederum die Ansicht über die schädliche CO-Wirkung beim Menschen

Zu Diagnose leichtere CO-Vergiftung

Durch unseren Hyperventilationstest im Liegen tritt schon bei 25%iger CO-Vergiftung, welche noch recht wenig Allgemeinsymptome macht, nach wenigen Sekunden ein kurzdauerndes Koma auf welches in wenigen Sekunden spontan behoben ist und nach unseren zahlreichen Eigenversuchen wegen der Kürze seiner Dauer als durchaus harmlos bezeichnet werden darf Dieser einfache Test soll die Blutanalyse nicht erdrängen, sondern im wesentlichen zur Bereicherung der *Frühdiagnostik* beitragen

Zur Frage der chronischen CO-Vergiftung

In der Literatur besteht verschiedentlich die Ansicht, dass es eine chronische CO-Vergiftung gäbe als Folge einer langen Kette leichter ‚Angiftungen so besonders durch Symansky (1936) Drinker (1938) Zorn (1965) u. a.

Der Begriff der chronischen CO-Vergiftung sollte nur auf solche Fälle bezogen werden und nicht auf chronisch gewordene Schädigung durch einmalige, starke CO-Vergiftung.

Mehrheitlich scheint nach dem internationalen Symposium der CO-Forschung von 1965 in Stuttgart die Meinung zu überwiegen wonach es eine echte chronische CO-Vergiftung durch unterschwellige CO-Belegung des Blutes nicht gebe. Ny-

ström (zit. bei Schirtek) konnte bei Ratten, die er über mehrere Monate auf einem CO-Hb-Spiegel von 10–20 % gehalten hatte, keinerlei histopathologische *Veränderungen feststellen*, desgleichen auch Ahlmark an Mäusen.

Die Vorstellung einer Vergiftung von Gewebskatalyten durch CO ist nach den früher vorgebrachten Argumenten zu verlassen, da die hierzu nötige CO-Spannung in solchen Fällen nie ausreichen würde.

Eine nur mittelstarke, immer wieder erfolgende CO-Vergiftung wird sich nach all dem Gesagten weniger während der Arbeitsleistung auswirken, wo praktisch nur die verstärkte Arbeitsdyspnoe (Muskelmilchsäure!) auffällt, als vielmehr in der darauf folgenden Ruhepause. Diese führt zur Reduktion der während der Arbeit sich im Blut angehäuften Muskelmilchsäure. Es kann dabei eine vorübergehende leichte Alkalose des CO_2-verarmten Blutes auftreten mit der beschriebenen schädlichen Wirkung im CO-Organismus. (Vergl. unsere eingangs geschilderten Hyperventilationsohnmachten bei leichtem Blut–CO-Befall.)

Je länger aber eine körperliche Anstrengung im Stadium *nach* leichterer CO-Vergiftung in frischer Luft anhält, desto rascher wird das im Blut verankerte CO durch die Muskelmilchsäure und die grosse Atmung wiederum ausgetrieben. Folglich sind Zustände nach nur kurzer Arbeitsleistung bei mässig stark CO-vergifteten Patienten unter Umständen gefährdender als nach länger anhaltender Arbeit (sofern die Personen sich wiederum in Frischluft bewegen).

Wie bei körperlicher Anstrengung führt auch die Zufuhr von CO_2 zu einem ruhenden Patienten mit nur mittelstarker CO-Vergiftung zu einer grossen Atmung mit baldiger subjektiver und objektiver Besserung der Symptome, wie wir das im Selbstversuch beschrieben haben. *Daraus ergibt* sich die bekannte Nützlichkeit der therapeutischen CO_2–O_2 Beatmung von CO-Patienten, auf welche *Haldane* und auch *Henderson* hingewiesen haben. Im grossen Gegensatz hierzu wäre jedoch eine passive forcierte *Hyperventilation* nach dem beschriebenen äusserst gefährlich.

Gibt es eine Gewöhnung an leichtere CO-Vergiftung?

Haldane hat durch seine während längerer Zeit täglich vorgenommenen Eigenversuche diese Frage bejaht, indem mit zunehmender Gewöhnungszeit die Symptome einer CO-Vergiftung später oder milder auftraten. Nach längerer Latenzperiode traten die CO-Symptome wiederum bei geringerer *CO-Konzentration auf*.

Im Eigenversuch konnte ich diese Beobachtung voll bestätigen. Nach einem 14tägigen Höhenaufenthalt im Forschungsinstitut Jungfraujoch (3450 m) musste ich zur Auslösung gleich starker subjektiver Störungen anstatt eines 5 %igen ein 7,5 %iges CO–Luftgemisch während 5 Minuten in Ruhelage einatmen. Zwar waren hierbei Schriftproben und Rechenaufgaben gleich gut möglich wie vorher nach Atmung von % CO mit 30 %iger CO–Blutsättigung. Jedoch kollabierte ich 2mal beim Versuch, frei herumzugehen. Dabei betrug mein Blut–CO-Gehalt 55 % CO.

Man kann sich also innerhalb gewisser Grenzen an CO-Blut-Befall ähnlich wie an O_2-Mangel bis zu einem gewissen Masse gewöhnen, was nach der neuen Erklärung, aber auch durch die Wirkung der 2,3-Phosphoglyzerate einfacher zu deuten ist.

Die in der Literatur gelegentlich gemeldeten Fälle repetierter CO-Vergiftung mit nur 2 % CO–Hb (z. B. Zorn), welche zu klinischen Erscheinungen führen, sind mit grosser Vorsicht aufzunehmen, wenn man sich an die Schwierigkeit genauer CO-Blutanalysen und an die Kriterien der korrekten Blutentnahmen erinnert und nicht zuletzt auch daran, dass die Symptome *nach* CO-Vergiftung sich noch verstärken können, zur Zeit, da der CO-Gehalt im Blute schon wieder im deutlichen Abnehmen begriffen ist. Haldane weist auf S. 203 darauf hin und unsere Eigenversuche mit dem oft sekundär *auftretenden Kopfschmerz nach* der CO-Atmung weisen ebenfalls in diese Richtung. Auch rät Symansky (1965), man solle keinesfalls nur eine einzige CO-Bestimmungsmethode anwenden, da man bei keiner völlig sicher sei.

Übrigens sprechen unsere beschriebenen Versuche mit völlig symptomloser Einatmung von 4,5 %iger CO-Luft (unreduziert) auf Jungfraujochhöhe mit einer Blut–CO-Sättigung von nur 12–15 % CO gegen die Vorstellung einer Schädigung von nur 2 % Blut–CO-Gehalt im Tal, da diese 2 % ja im obersten Teil der Haemoglobin–O_2-Sättigungskurve eintreten würden, die schon normalerweise nicht durch O_2 belegt sind.

Hyperventilationsversuch an CO-Meerschweinchen

Unsere früher besprochene k

ergab sich in Bezug auf das erste Herumgehen dieser primär druch 20% CO-Luftgemisch komatös gewordenen Tiere dass die kürzeste Erholungszeit in 20° Luft auftrat (19'45 als Durchschnittswert), die weitaus längste in 4 Luft (49'15") und eine mittlere in 37° Luft (28)

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Zur Frage der chronischen CO-Vergiftung

In der Literatur besteht verschiedentlich die Ansicht dass es eine chronische CO-Vergiftung gäbe als Folge einer langen Kette leichter „Angiftungen" so besonders durch Symansky (1936) Drinker (1938) Zorn (1965) u. a.

Der Begriff der chronischen CO-Vergiftung sollte nur auf solche Fälle bezogen werden und nicht auf chronisch gewordene Schädigung durch einmalige starke CO-Vergiftung.

Mehrheitlich scheint nach dem internationalen Symposium der CO-Forschung von 1965 in Stuttgart die Meinung zu überwiegen wonach es eine echte chronische CO-Vergiftung durch unterschwellige CO-Belegung des Blutes nicht gebe. Ny-

Punkt gestartet werden anstatt bei 25 50 oder 75% der CO-Vorsättigungswerte

Bei der menschlichen CO-Vergiftung dringt nun das CO vom oberen Ende der Blutsättigungskurve her ein, weil hier der Verdrängung des O_2 der geringste Widerstand entgegen gebracht wird. Das ist auch der Grund dafür dass in der Höhe eine CO-Ruheatmung im Liegen solange keine Symptome auslöst, als nur die oberste O_2-freie Partie der Blutsättigungskurve durch das CO beschlagnahmt *wird* Erst bei weiterer CO-Sättigung treten durch progressives Austreiben von O_2 aus den Erythrocyten Symptome von Pulsbeschleunigung und späterer Dösigkeit auf

Neu ist auch die Tatsache des rascheren Komaeintrittes bei Hyperventilation in der Höhe als im Tale bei gleichen Graden von CO-Blutsättigungen Dies wird auf einen spezifischen, funktionellen, cerebralen Alkaloseschaden bezogen Dieser wird verstärkt durch die hyperventilatorisch starke Linksverschiebung der O_2-Blutbindungskurve welche wiederum mit moderner spektrographischer Methode bestimmt *nach oben progressive Linksverschiebung aufweist*

Diese neue Tatsache erklärt unsere Versuche welche trotz konstantem Blutdruck bei stärkster Hyperventilation in kurzer Zeit zur Bewusstlosigkeit führten zusammen mit der du ch die Alkalose bedingten *cer bralen* Erschwerung der O_2-Verwertung.

Die neue Auffassung der herabgesetzten O_2-Spannung und Menge im CO-haltigen Blute erklärt viel besser die gewaltigen Unterschiede im Verhalten von Menschen mit Anaemie von 50% Hb (welche noch arbeitsfähig sind!) und bei 50%-iger CO-Blutsättigung welch letzteres einen Zustand an der äussersten Grenze eines nur noch im Liegen erhaltenen Bewusstseins darstellt.

Es wird ein Eigenversuch mit 55%iger Blut-CO-Sättigung beschrieben welcher zwar im Liegen zu zitteriger aber fehlerfreier Schrift führte jedoch wiederholtes Kollabieren mit Bewusstseinsverlusten bei Geh-Versuchen bedingte

Daneben wird durch Tierkurzversuche (Kaninchen und Meerschweinchen) gezeigt, dass nicht das Herz, sondern zuerst das Vasomotorenzentrum und hernach das Atemzentrum durch eine akute CO-Vergiftung gelähmt werden. Deshalb können solche im Schnellversuch bewusstlos gewordene und völlige Atemlähmung aufweisende Tiere durch sofortiges E nbringen in eine kleine Überdruckkammer bei 2-3 Atm O_2-Druck in sehr kurzer Zeit wieder zum Bewusstsein gebracht werden da auf diese Weise O bei noch *schlagendem* Herzen in die Lungen gepresst wird und über den rein physikalischen Weg zum bald wiederum normal werdenden Leben genügt. Dabei helfen neben dem erheblichen O_2-Überdruck auch die während des längeren Atemstopps auftretende starke hypoxische Blutübersäuerung zum erstaunlich raschen Austreiben des CO Deshalb können solche Tiere nach wenigen Minuten der O_2 Überdruckatmung wiederum symptomlos ausgeschleust werden.

Die höhere Empfindlichkeit *kleinere* Säugetiere (Mäuse) gegenüber der CO-Vergiftung wird auf den erhöhten Grundumsatz kleinerer Tiere zurückgeführt Interessant ist ferner die Tatsache dass bei narkotisierten tracheotomierten Kleintieren (Kaninchen) die registrierten Atemkurven beim Übergang zu 5–10%iger CO-Atmung ohne orherige Anzeichen urplötzlich sistieren wie dies auch nach i v Na-Nitrit Injektion geschieht.

Die klinisch so grosse Mannigfaltigkeit der Symptome nach einer CO-Vergiftung scheint nicht mehr so verworren wenn man sich vorstellt dass nicht alle Partien des ZNS gleichermassen durch Blut irrigiert werden Da wo aus irgendeinem Grunde eine auch nur geringfügige Hypozirkulation vorbesteht wirkt sich der hypoxische Schaden eben am ersten und stärksten aus

Unser Hyperventilationstest bei frischen fraglichen und leichteren Fällen on CO-Vergiftung wird als neues einfaches Hilfsmittel zur Früherfassung der Diagnose orgeschlagen Ein möglichst frühzeitiges Einbringen eines CO-vergifteten Menschen in 3 Atm O_2-Druck entspräche der bisher wirksamsten Therapie

Am Schluss sei meiner treuen wissenschaftlichen Laborantin Frau L. Buchler herzlichst gedankt für ihre während nahezu 30 Jahren in vorbildlicher Art geleisteten Dienste

LITERATUR

Gesamtüberblick über Literatur der CO-Vergiftung siehe im „Internat. Symposium Kohlenmonoxyd-Intoxikationen Arbeitsmed. Soz Med und Arbeitshygiene Heft 1 1965

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CO-Versuche ergaben jeweils den initialen Atemstop der bei nachfolgender Luftzufuhr allmählich unterbrochen wurde durch immer häufiger auftretende grosse Schnappatemzüge

Es interessierte uns nun die Frage ob bei diesen vornarkotisierten CO-Tieren durch passive Hyperventilation gegen Luft auch die beim Menschen beschriebene schädliche Hyperventilations-alkalose-Wirkung zu erzielen wäre

Die Resultate meines Dissertanden *Streit* verliefen jedoch negati desgleichen auch wenn nach dem initialen CO-Atemstop eine verdünnte NaOH Lösung sofort i v nachgespritzt wurde Damit scheinen narkotisierte Meerschweinchen welche übrigens gegen O_2-Mangel ohnehin schon viel resistenter sind als der Mensch diesen gefährlichen Effekt der Alkalosewirkung bei CO-Vergiftung nicht aufzuweisen

Zur O_2-Therapie der CO-Vergiftung

Haldane und auch Henderson haben mit Recht auf die Wichtigkeit der CO_2-Zugabe zur therapeutischen O_2-Atmung bei CO-Patienten hingewiesen weil dadurch ein viel grösseres Atemvolumen und raschere CO-Austreibung erfolgen. Neuerdings schlug jedoch Kreuzer am internalen CO-Symposium 1965 aus theoretischen ... or die O_2-Atmung bei CO-Ver... nicht mit einer CO_2-Atmung, sondern im Gegenteil mit einer Hyperventilation zu kombinieren was jedoch nach unseren beschriebenen Versuchen gefährlich sein kann wenn es nämlich im Verlaufe zur Hyperventilations-Alkalose käme

Die eben beschriebenen Versuche mit der so raschen Erholungszeit akut CO-vergifteter Tiere durch O_2-Überdruck *würden diese Therapie* auch für den Menschen empfehlenswert erscheinen lassen. Jedoch würde dies nur für allererste Phasen der Vergiftung von grossem Wert sein also für Fälle welche noch nicht lange in tiefster Bewusstlosigkeit gelegen haben. Bei allen andern konnen unter Umständen hypoxische Schädigungen so weit vorgeschritten sein dass irreparable ornehmlich cerebrale Ausfälle stattgefunden haben. Dies selbst in Fällen, wo im Blut (anlässlich der Spitaleinweisung) dann nur noch Spuren on CO vorliegen, worauf Haldane schon hinwies. So gelang es uns nicht im Jahre 1945 eine CO- ergiftete Frau on 40 Jahren welche eine ganze Nacht lang in einem CO-vergasten Zimmer bewusstlos lag durch Einbringen in unsere O_2-Überdruckkammer mit 3 Atm O_2 zum Bewusstsein zu bringen Wohl stieg der Blutdruck an doch starb die Patientin 12 Stunden nach der halbstündigen O_2-Überdruck-Behandlung ohne das Bewusstsein wiederum erlangt zu haben. Ein guter aber trauriger Beweis dafür dass unter der langen Hypoxie eben irreparable Schädigungen vornehmlich cerebraler Art stattfinden können.

Als Analogen hierzu bekam eine 60jährige Frau 4 Tage nach einer Struma maligna-Operation bei welcher ich die Carotis communis (die durch den Tumor umwachsen aber intakt war) erhalten konnte (und musste!) eine Spontanruptur dieser nicht mehr durch Tumor umwachsenen muskelschwach gewordenen Carotis Nach bald erfolgender Blutersatz Infusion und Bluttransfusion in die rupturierte nicht mehr blutende Carotis gelang es zwar den Blutdruck wieder zu normalisieren, jedoch blieb die Patientin bewusstlos und starb nach 36 Stunden Hier war das Gehirn ebenfalls irreversibel geschädigt In gewisser Beziehung ein Analogon zum irreversiblen CO-Gehirnschaden.

1965 haben Goulon u Mitarbeiter über 20 Fälle on CO-Vergifteten die i einer O_2-Überdruckkammer behandelt wurden berichtet. Diese Behandlung brachte in 13 Fällen komplette Heilung.

Zusammenfassung

Unsere frühere von vielen Klinikern gehegte Ansicht einer spezifischen CO-Giftwirkung auf den Menschen veranlasste uns anhand der in der Literatur beschriebenen Hemmung der Carbonhydrase durch CO an dieses Problem heranzutreten Demnach dürften mittelstark CO-vergiftete *Versuchspersonen bei Ruhehyperventilation keine* oder stark verspätet auftretende Atmungstetanie aufweisen

In 12 freiwilligen Versuchen mit jeweils 25–30%iger Blut–CO-Sättigung traten in Liegelage jedoch unerwarteter Weise schon nach 6–10 tiefsten und raschen Atemzügen kurze Bewusstlosigkeiten auf – Dies ist nach den Haldane schen CO–O_2-Blut Sättigungskurven in Verbindung mit den durch CO_2-Verlust bedingten Bohrkurven nicht genügend zu erklären.

Zunächst wird auf eine irreführende Darstellung der CO–O_2-Blutbindungskurven im Buche on Haldane hingewiesen, wo trotz verschiedener Grade on CO-Vorsättigung die nachfolgenden O_2-Sättigungskurven immer bei Null auf dem gleichen

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Haemodynamic studies at rest and during exercise in patients treated with artificial pacemaker

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The present publication is based mainly on studies reported in the following papers:

I. Effect of changes in ventricular rate on cardiac output and central pressures at rest and during exercise in patients with artificial pacemakers.
Bevegård, S., Jonsson, B., Karlöf, I., Lagergren, H. and Sowton, E.
Cardiovasc. Res. 1: 21, 1967

II. Effect of changes in ventricular rate on forearm bloodflow and systemic arterial pressure in patients with artificial pacemakers.
Bevegård, S., Karlöf, I. and Resnekov, L.
Scand. J. clin. Lab. Invest. 32: 245, 1973

III. Adaptation of the left ventricle to sudden changes in heart rate in patients with artificial pacemakers.
Karlöf, I., Bevegård, S. and Ovenfors, C.-O.
Cardiovasc. Res. 7: 322, 1973

IV. Atrial-triggered pacemaking without thoracotomy. Apparatus and results in twenty cases.
Lagergren, H., Johansson, L., Karlöf, I. and Thornander, H.
Acta Chir. Scand. 132: 678, 1966.

V. Haemodynamic effect of atrial triggered versus fixed rate pacing, at rest and during exercise, in complete heart block.
Karlöf, I.
Acta Med. Scand. 1974. In press.

References to these papers in the text will be made with the Roman numerals I—V.

Contents

Introduction

The treatment of syncope with a galvanic current was first suggested by Aldini in 1819 (2). Ten years earlier (1809) Burns hade noticed that the heart could be stimulated by electricity (35). In 1932, Hyman published a report describing a pacemaker which was able to control the heart rate in experimental animals (21). He also suggested that it could be made portable and used in patients. Callaghan and Bigelow (1951) described successful experiments with electrical stimulation in the region of the sino-atrial node with a bipolar needle (7). The experiments were performed in animals with an intact conducting system. Zoll in 1952 was the first to successfully treat Adams-Stokes attacks with an artificial pacemaker (42). He applied electrodes externally to the chest wall. As this type of pacing needs high voltages, and therefore is painful, because of strong contractions in the muscles in the thoracic wall, attempts were made to use more direct stimulation. Thevenet, Hodges and Lillehei 1958 stimulated the heart with a much lower impulse amplitude by means of an electrode introduced trans-thoracically (38). The threshold for electrical stimulation increased, however rather soon because of formation of fibrous tissue around this electrode. The same year Furman and Robinson (17) reported that stimulation of the heart could be achieved with an electrode catheter introduced transvenously into the right ventricle. As a treatment for complete AV block which occurred during open heart surgery Weirich et al. (39) 1958 sutured electrodes on the myocardium and connected them to a small portable external pacemaker. In order to avoid wires passing out through the skin, with possibilities of infections following the cables to the heart, two fundamentally different types of implantable pacemakers were constructed. One was the inductive coupled pacemaker in which one coil was positioned subcutaneously and the other superimposed externally and connected to the impulse generator (1, 8, 19, 34, 41). The other was the completely implantable pacemaker first developed by Elmqvist and implanted by Senning 1958 (16, 33). As energy source this unit had a pair of rechargeable, nickel-cadmium cells. In 1960 Chardack, Gage and Greatbatch (12) described an implantable pacemaker with mercury cells, and since then, this has been the dominating energy source for pacemakers (15, 22, 28, 43). Implantable pacemakers, which usually give impulses at a fixed rate of around 70/min, have since then been improved throughout the years. In the beginning the impulses were transmitted to the heart with electrodes sutured to the epicardium, an operation which required thoracotomy. In order to avoid this major operation in patients who often were old and in a bad condition. Furman transvenously introduced an electrod catheter to the outflow region of the right ventricle (18). This method was further developed by Lagergren and Johansson who used an electrode with a very soft cabl constructed by Siemens-Elema AB (24). This electrode was easily passed to the apical region of the right ventricle, the threshold for stimulation remained low and the cable did not break, a complication which was rather common with earlier electrodes.

I 1962 Nathan and Center implanted for the first time a pacemaker in which the impulses to the ventricles were *triggered by the atrial activity* (27). The P waves, picked up by an electrode sutured to the left atrium, triggered the pacemaker to give an impulse after an delay. In this way the pacemaker normal time relation between atrial and contraction, and the rate according to metabolic

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a patient for the first time in 1965 (10). The atrial electrode of this pacemaker was introduced by means of mediastinoscopy (9). In this way a *patient could receive an atrial triggered pacemaker* without running the risks of a thoracotomy.

At about the same time many manufacturers started to construct pacemakers with the ability to sense the ventricular activity. These were designed for patients with unstable AV-block, and have two principally different modes of function. One *type is completely inhibited by the QRS complex*, the other is triggered by the R wave to give a physiologically ineffective impulse in the QRS complex, as long as the patient is in sinus rhythm. In case of complete block, these pacemakers give impulses of a fixed rate to the right ventricle via the same electrode which senses the ventricular activity. An R wave synchronous pacemaker was designed by Siemens-Elema AB in 1967 with a construction principally based on the atrial triggered pacemaker (23).

The haemodynamic effect of pacing at a fixed impulse-rate both at rest and during exercise has been studied by many authors (3, 4, 20, 26, 29, 30, 32, 36, 37). With the development of pacemakers with different modes of function the indications for the different types had to be further analyzed. Was the haemodynamic benefit to a certain patient receiving an atrial triggered pacemaker so great that it could outweigh the possible disadvantages of *giving him a more complicated and* expensive unit? Or were there in some patients contraindications for one type of pacemaker? The beneficial haemodynamic effect of atrial triggered pacing has been emphasized among others by Braunwald et al., Brockman et al., Center et al., Martin et al., and Samet et al. (5, 6, 11, 25, 31). *To the author's knowledge there is, however, no* study in which the haemodynamic effect of atrial triggered versus fixed rate pacing has been investigated in the same patients during exercise on fairly high work loads.

The present study is in its first part an attempt to analyse firstly the haemodynamic effects of fixed rate pacing at different rates at rest and during work, secondly the immediate effect of sudden changes in rate. The second part of the study is devoted to atrial triggered pacing. The haemodynamic effects of a synchronized atrial systole per se as well as the effects of treatment with an atrial triggered versus a fixed rate pacemaker are analysed. Finally the indications and contraindications for treatment with atrial triggered pacemakers are discussed.

I. HAEMODYNAMIC STUDIES AT A CIRCULATORY RELATIVE STEADY STATE

Twenty-two patients with complete AV-block treated with artificial fixed rate pacemakers were studied in paper I. There were three women and nineteen men between the ages of 14 and 78 years, mean 52. Pacing was carried out with an intravenous electrode catheter previously placed in the apical region of the right ventricle (24) except in two patients, in whom epicardial electrodes were used (15). The indifferent electrode was positioned subcutaneously below the left costal arch. The cables were connected to an external pacemaker (Siemens-Elema, model EM 138), the impulse frequency of which could be varied between 30 and 170 impulses/min. The patients were investigated with heart catheterization in supine at different heart rates at rest and during exercise.

Effect of different pacing rates at rest

Cardiac output. When the ventricular rate was changed from idioventricular to a normal resting level of about 70 beats/min, cardiac output increased, but then remained constant despite a further rate increase of up to about 160 beats/min.

Within the range of ordinary heart rates at rest, the cardiac output was normal or somewhat small in relation to the oxygen uptake. This means that the arteriovenous oxygen difference was normal or somewhat increased. In one patient who had mitral incompetence the arteriovenous oxygen difference was very high, even at normal heart rates.

Stroke volume. The stroke volume was largest at idioventricular rate and decreased continuously with increasing ventricular rate.

Oxygen uptake. When the ventricular rate was increased from an abnormally low to a normal level, the oxygen uptake did not change significantly.

Intracardiac and intravascular pressures. The right ventricular filling pressure was abnormally high at very low ventricular rates, but decreased to normal levels when heart rate was increased. In the patient with associated mitral incompetence, the filling pressure, however, remained high despite increase in ventricular rate.

The pulmonary artery mean pressure was normal at low heart rate in most patients, and only slightly increased in a few. It remained almost unchanged at higher ventricular rates. The only exception was the patient with mitral incompetence in whom this pressure was moderately increased at low ventricular rates and showed a further marked increase at higher rates. The initially low aortic mean pressure was normalized, when the ventricular rate was altered from very slow to normal levels, around 70 beats/min. A further increase in rate caused an additional slight rise in pressure.

Peripheral vascular resistance did not change with variation of ventricular rate.

Effect of different pacing rates during exercise

In 15 patients the effect of exercise was studied at paced rates between 56 and 152 beats/min. During exercise at a fixed ventricular rate cardiac output increased in all patients, but was usually lower than normal in relation to oxygen uptake. Consequently stroke volume increased with increasing work load. In many patients there was a considerable rise in the right ventricular filling pressure and in pulmonary arterial pressure. The

aortic pressure increased normally in most patients.

Nine patients were exercised at levels close to their maximal capacity. At rates up to 110 beats/min, the stroke volume in most patients increased approximately to the value obtained at rest during idioventricular rhythm.

Three patients, who were exercised at rather high work loads at ventricular rates between 120 and 150 beats/min, were not able to attain their maximal stroke volumes. In these patients cardiac output did not increase further when the ventricular rate was raised above 110 beats/min.

In five patients the ventricular rate was altered between 56 and 109 beats/min, during exercise on a constant rather low work load. The stroke volume and right ventricular filling pressure decreased with increasing ventricular rate, and the cardiac output increased only slightly.

Conclusions

1. In patients with complete heart block treated with artifical unsynchronized ventricular pacing, cardiac output at rest increases when the ventricular rate is increased from slow idioventricular rate to 70 beats/min, but remains rather unchanged with a further increase in rate up to about 160 beats/min. During exercise cardiac output increases, in spite of a fixed ventricular rate, owing to an increase in stroke volume. Above a certain lower limit in ventricular rate the cardiac output is regulated by metabolic demands, relatively independent of further artificial variations in rate. This implies that no marked increase in cardiac output can be obtained by increasing ventricular rate in these patients neither at rest nor during work.

2. Above a certain lower rate, stroke volume varies inversely with ventricular rate. This is true both at rest, and during exercise on a constant work load.

Judging from the rather few patients studied during heavy work, a maximal stroke volume can be maintained up to a ventricular rate of the order of 110 beats/min. Above this rate stroke volume is decreasing.

3. At very low ventricular rates, aortic pressure is low and the right ventricular filling pressure high. Both are more or less normalized when the ventricular rate is increased into the normal range of about 70 beats/min.

4. The investigation confirms that an implantable fixed rate pacemaker should give impulses at a rate around 70 impulses/min. If an implantable asynchronous pacemaker with variable frequency is constructed, its maximal rate could probably be around 110 impulses/min, if only an increase in cardiac output is intended.

II. HAEMODYNAMIC STUDIES OF SUDDEN CHANGES OF RATE

In paper I the responses of the aortic pressure and ventricular filling pressures, after a sudden increase of unsynchronized ventricular rate, were studied in 9 patients. The results obtained showed rather large, individual variations, but suggested that marked transient changes in blood flow may occur when a sudden change in ventricular rate is produced. Paper II is an attempt to get more information about the beat to-beat adaptation of the circulation, following sudden changes in unsynchronized ventricular rate. The studies in this paper offer however only indirect information about the beat to-beat changes in stroke volume. In paper III the sudden changes in stroke volume are studied more directly by measuring the angiocardiographic volume changes of the left ventricle, following sudden alterations in ventricular rate.

Effects on forearm blood flow, forearm pulse volume and brachial artery pressure

The effect of sudden changes in heart rate at rest on forearm blood flow, using strain-gauge plethysmography and brachial arterial pressure, was studied in six patients being treated with external pacemakers for complete heart block (II). The age of the patients ranged from 55 to 77 years and none of them had any clinical or radiological evidence of cardiac decompensation at the time of the study. The amplification used for measuring forearm blood flow permitted increases in the forearm volume of each beat to be measured when

a plateau had been reached for each pulse. Ventricular rate was changed suddenly from a low idioventricular to a normal rate of 70 beats/min, from 70 to 150 beats/min and from slow idioventricular to 150 beats/min.

Immediat ffects The forearm blood flow was relatively unchanged when ventricular rate was varied within broad limits, although sudden increase of rate, from idioventricular (27—49 beats/min) to a paced ventricular rate of 70 beats/min on the average, caused a minor but not significant increase in forearm blood flow A sudden increase in ventricular rate was associated with a decrease in forearm pulse volume in all patients. Arterial pulse pressure likewise decreased in all but one patient, when ventricular rate was changed from idioventricular to 70 beats/min. An immediate decrease in ventricular rate was followed by immediate increases in forearm pulse volume and arterial pulse pressure. In each instance these changes in pulse-pressure amplitude and pulse volume occurred from one beat to the next, and appeared proportional to the diastolic filling time. Furthermore a beat-to-beat analysis showed a close correlation between changes in amplitude of the arterial pulse pressure and changes in forearm pulse volume.

Lat effects A further three to five measurements of forearm blood flow were made in a 90 second period, after changing ventricular rate. During this time when the rate was changed from slow idioventricular to 70 beats/min, transient increases in forearm blood flow were found in some patients, whereas flow stabilized within one minute on a sl ghtly higher level. The difference was, however not statistically significant. In one patient whose idioventricular rate was rather high (42 beats/min) no change in flow was recorded

When ventricular rate was changed suddenly from 70 to 150 beats/min, transient decreases in forearm blood flow occurred in four patients, but by the end of the 90 second period the flow had returned to approximately the same level as before the rate change. In one patient, however, the flow was almost unchanged during the observation period.

When ventricular rate was changed from 30 to 150 beats/min, both transient increases and transient decreases in forearm blood flow were measured, but thereafter a new stable level of forearm blood flow was established.

Conclusions

1 Sudden changes in ventricular rate cause only minor and non-significant changes in forearm blood flow when measured immediately after and within approximately one minute from the change of rate.

2. Forearm pulse volume varies inversely with the ventricular rate in proportion to diastolic filling time, and the changes in pulse volume occur from one beat to the next. Presumably the changes in forearm pulse volume reflect changes in left ventricular stroke output.

Effects on left ventricular volume

Five patients, two women and three men, between the ages of 48 and 74 years were studied. All five patients had complete heart block of unknown origin One of them also had an aortic stenosis (systolic pressure difference 50 mm Hg) They were treated with an external artificial pacemaker (Siemens-Elema, model EM 138) i which the impulse frequency could be varied between 30 and 150 impulses/min. The impulses were transmitted to the heart via a transvenous monopolar electrode, the tip of which was positioned in the apical region of the right ventricle. The patients were investigated in the supine body position. Aortic pressure was recorded through a catheter the tip of which was positioned in the ascending aorta. One plane cineangiocardiography was performed with injection of contrast medium in the right atrium, and exposures made at a rate of 42 frames/s in the right anterior oblique projection During the passage of contrast through the left ventricle, the ventricular rate was suddenly altered by changing the impulse frequency of the pacemaker The inner volume of the left ventricle was calculated regarding the left ventricle as a rota

tional ellipsoid with concentric contractions. In one of the patients, who at the time of the study showed interference between sinus rhythm and pacemaker induced rhythm, no alteration in pace maker rate was performed.

Stroke volume In patient no. 1 the ventricular rate was changed from an idioventricular rhythm of 45 beats/min to a pacemaker induced rate of 100 beats/min. The stroke volume calculated from the left ventricular volume changes decreased immediately from 135 to 75 ml. In spite of the decrease in stroke volume, the cardiac output calculated from the left ventricular volume changes and the heart rate increased slightly At a circulatory steady state after 15 min with a heart rate of 100 beats/min, the cardiac output calculated from left ventricular volume variations was unchanged, compared with the cardiac output calculated at a ventricular rate of 45 beats/min. When the pacemaker was suddenly switched of in this patient, the heart rate fell instantaneously from 100 to an idioventricular rhythm of 27 beats/min, and stroke volume increased momentarily from 60 to 120 ml.

Patient no. 2 had initially an idioventricular rate of 35 beats/min when the pacemaker was suddenly switched on at a rate of 140 impulses/min. During the first beats, however the heart was stimulated only by every other impulse resulting in a heart rate of 70 beats/min Approximately 15 min later the same procedure was repeated. This time the heart was activated by each pacemaker impulse, and the rate suddenly increased from 35 to 140 beats/min. At a change in heart rate from 35 to 70 beats/min, the duration of mechanical diastole at the higher rate still permitted unchanged diastolic filling of the ventricle during the first four beats. A twofold increase in heart rate and mainly constant stroke volume increased the calculated cardiac output from 3 5 l/min to 8 0 l/min, while the amplitude of the aortic pressure curve decreased somewhat. When the heart rate was suddenly changed from 35 to 140 beats/min, the stroke volume was reduced from one beat to the next to about one half giving a calculated cardiac output of 7 7 l/min, which was of the same order as the cardiac output at a heart rate of 70 beats/min. In patient no 3 the pacemaker induced ventricular rate was changed from 40 to 70 and from 70 to 100 beats/min, which gave an immediate change in stroke volume from 82 to 70 and from 67 to 50 ml respectively In patient no 5 the steps in rate were from 50 to 100 and from 100 to 50 beats/min giving immediate stroke volume changes from 90 to 60 and from 60 to 105 ml respectively

This direct study of the left ventricular volume variations confirms the observations in papers I and II i.e. that stroke volume and pulse volume of the arm vary inversely with heart rate. It also confirms that the change in stroke volume occurs from one beat to the next following a sudden change in rate. This is, however true only within certain rate limits It is obvious that in each subject there should be a lower limit for pulse rate, below which the heart can no longer compensate for the low rate by an increase in stroke volume, in order to keep cardiac output constant. When the heart rate is increased from a low level, stroke volume will initially remain unchanged up to a certain rate, where further shortening of the diastolic filling period will result in an inverse decrease of stroke volume with increase in heart rate. The level of this lower critical heart rate depends on such factors as myocardial compliance and available filling energy for the ventricles. This reasoning is examplified by the study mentioned above in which an increase in ventricular rate from 35 to 70 beats/min caused no change in stroke volume during the first four beats recorded. According to the earlier studies, papers I and II, a decrease in stroke volume could be expected after these first beats probably depending upon a decrease of available filling energy due to decreased filling of capacitance vessels close to the heart. This transient increase of cardiac output, when heart rate is changed from a low to a normal level, is indirectly demonstrated by the repeated determinations of forearm blood flow (II)

End-diastolic and end systolic volume In the four patients mentioned above the alterations in

stroke volume were brought about by changes in end-diastolic ventricular volumes. The end-systolic ventricular volumes on the other hand were mainly constant in each patient, although the changes in heart rate and stroke volume were large. This was true also for patient no 4, who at the time of the study showed interference between sinus rhythm and pacemaker induced rhythm. Here no alteration in pacemaker rate was performed. The study just shows ventricular volume changes during sinus beats and pacemaker induced beats. In spite of the different origin of the left ventricular activation, the volume changes were approximately the same, and the end systolic volumes mainly constant.

The principal factors determining the left ventricular end-diastolic volume seem to be the duration of mechanical diastole, the available filling energy during diastole and the elastic and esistive properties of the myocardium.

Conclusions

1 When heart rate is changed above a certain lower limit, left ventricular stroke volume varies inversely with heart rate.

2. The alterations in stroke volume are brought about by changes in left ventricular end-diastolic volume These changes are proportional to the duration of mechanical diastole and filling energy The end-systolic volume remains unchanged.

3 Left entricular end-diastolic volume and stroke volume adapt, from one beat to the next, to changes in heart rate.

4. The results indicate that there is a lower border frequency at which the ventricular end diastolic volume is maximal. Below this frequency a further increase in length of diastole does not increase the stroke volume, and cardiac output then decreases in proportion to the decrease in heart rate.

B Atrial triggered ventricular pacing

I. Apparatus (IV)

Before the atrial triggered pacemaker could be constructed, the possibilities of deriving a P wave of sufficient amplitude from the atria and of using that P wave to trigger an artificial pacemaker, had to be studied. For this reason, an apparatus for cardiac resuscitation manufactured by Corbin Farnsworth Inc. Palo Alto Calif was modified in 1963 After the modification the apparatus could give atrial triggered pacemaker impulses and the following parameters could be varied input sensitivity delay between input signal and impulse delivery blocking time and basic rate. With this device the appropriate properties for an atrial triggered pacemaker were determined, and based on the collected data such a pacemaker was constructed by Siemens-Elema AB In 1965 the first pacemaker was implanted in a patient. Since then the apparatus has undergone several stages of development, the principal data are, however unchanged. The current source comprises 4 mercury cells and the impulse voltage is about 5.4 V with a pulse duration of 2 ms (on later models decreased to about 1 ms) The pacemaker has a fixed basic rate of 50 impulses/min (on one model 60 impulses/min) and a highest synchronous rate of 150—170 impulses/min. It can be triggered by a P wave with a lowest amplitude of 0.9 mV (on a special model 0.4 mV) and the delay of the stimulating impulse is approximately 0 ms. The bandwidth of the P wave amplifier has been limited from 5 to 100 Hz in order to make the device less sensitive to external electro-magnetic interference. The output and input circuits have Zener diodes in order to decrease the risk of pacemaker damages, in case of external counter shock. When the atrial rate approaches the pacemaker's highest synchronous rate every 4th or 5th impulse drops out With a further rise in atrial rate, a constant 2 1 block appears.

II. IMPLANTATION OF THE ATRIAL DETECTOR ELECTRODE

P waves of an amplitude sufficient to trigger the pacemaker are obtained by an electrode positioned in close contact with the atria. This electrode is introduced as follows without thoracotomy by means of mediastinoscopy (9) Under general anaesthesia with the patient intubated, after administration of a muscular relaxant, a small incision is made in the jugular fossa. After perforation of the pretracheal fascia, dissection is performed by the aid of a finger along the trachea in the loose tissue to the front. The mediastinoscope is then introduced. Dissection is continued, under visual control, beyond the bifurcation of the trachea and behind the right branch of the pulmonary artery to the thin layer of connective tissue between the posterior wall of the atria and the oesophagus. The electrode tip is then placed, with the aid of a forceps, in the connective tissue as close as possible to the atrial wall. The amplitude of the P wave, led off by the electrode is checked on the electrocardiogram during the procedure. After a P wave amplitude of at least 1.0 mV has been obtained, the electrode is left in this position the only fixation consists of suturing the cable in a few loops below the muscles of the neck. The rest of the cable is then passed subcutaneously to the right groin with the help of a wide-bore injection needle Here the cable is passed out through the skin, in order to make it possible to check the amplitude of the P wave for some time during healing The mediastinal electrode is of the same design as the electrode used for transvenous intraventricular pacing (21) It consists of a soft,

flexible cable, 1.5 m long and 1.2 mm in diameter. The cable has a core of Terylene around which four thin strips of fatigue-resistant stainless steel are wound. It has an insulating covering of polythene. A cylindrical electrode tip of platinum is attached to one end of the cable.

III. HAEMODYNAMIC STUDIES OF ATRIAL TRIGGERED PACING

Twentyfive patients with complete AV-block were studied using fixed rate and atrial triggered pacemakers (V). In all patients a transvenous electrode (Siemens-Elema, EMT 588) had previously been introduced to the apical region of the right ventricle, and an indifferent electrode had been placed in the fatty tissue of the abdominal wall. A P wave sensing electrode had been implanted by means of mediastinoscopy in each patient except one. In this patient the P wave was obtained from a temporary oesophageal electrode. The cables of the implanted electrodes were passed out through the skin in the right groin. The study consists of two series. In the first, including 12 patients, central pressures and cardiac output were measured at a fixed rate pacing of about 70 impulses/min, FRP and at atrial triggered pacing, ATP. Most patients were studied both at rest and during exercise, the work loads being identical with both types of pacemakers. In the second series 13 patients were studied at rest and during exercise. The measurements of pressure and cardiac output were first performed with the patients on ATP. After a 30 min rest an identical study was performed with the patient connected to a fixed rate pacemaker, the rate of which was matched, FRPm, to the same as had previously been recorded with ATP.

This design of the investigation makes series I the practical clinical part of the study. The connection of the atrial triggered pacemaker implies, not only synchronization of atrial and ventricular activity but also a normal increase in heart rate during exercise as compared to the fixed pacemaker's asynchronous rate of about 70 impulses/min. Series II, on the other hand is an attempt to study the effect of the synchronous atrial systole per se, the effect of rate being eliminated by adjusting the fixed rate pacemaker to the same rate as that obtained with the atrial triggered unit under the same conditions.

Effects of synchronized atrial systole

In series II, cardiac output with FRPm was on the average 4.5 l/min at rest, and increased during exercise to 8.5 l/min. The cardiac output under the same conditions with ATP averaged 5.3 l/min and 9.2 l/min respectively.

The heart rate with ATP averaged 77 beats/min at rest and 122 beats/min during work. With FRPm, interference rhythm occurred in three patients and the resulting heart rates were on the average 80 and 121 beats/min respectively. The resulting average stroke volume is significantly larger with ATP (69 ml) compared to FRPm (57 ml) at rest ($P < 0.01$). During work it is also larger with ATP but not significantly (77 ml compared to 72 ml). The filling pressure of the left ventricle, as reflected by the mean pulmonary artery wedge pressure or diastolic pulmonary artery pressure, was on the average lower with ATP significant on the 5 % level at rest, whereas no difference was observed during exercise.

These data indicate that a normal timing of the atrial and ventricular contraction as obtained with ATP gave on the average an 18 % higher cardiac output at rest and 8 % higher during exercise compared to FRPm. It is somewhat remarkable that the effect of ATP on cardiac output is more marked at rest than during exercise. Furthermore the stroke volume is not significantly larger with ATP and there is no difference in filling pressure between ATP and FRPm during exercise. With increasing heart rate and shortening of diastole, the atrial contraction should have a greater importance than at rest for the diastolic filling of the ventricles. There can, however, be many factors explaining the moderate effect of ATP. One important factor can be that the work loads were not at all maximal: on the average 409 kpm/min. This resulted in rather low average pulse-frequencies and conse-

quently the time for diastolic filling of the ventricles was rather long. Another factor is the marked individual variations. Some of the patients had an increase of cardiac output of more than 20 % with ATP compared to FRPm during exercise.

Effects of atrial triggered versus conventional fixed rate pacing on central haemodynamics

In series I, the effect of treatment with an atrial triggered pacemaker ATP compared to that of a fixed pacemaker at a rate of about 70 impulses/min, FRP is studied both at rest and during exercise. The cardiac output with FRP was on the average 5.2 l/min at rest and increased to 8.2 l/min during exercise, at an average work load of 383 kpm/min. With ATP the average cardiac output was 5.7 l/min at rest and 9.8 l/min during work. Thus ATP gives a 10 % higher cardiac output ($P < 0.02$) at rest and a 20 % higher output during work ($P < 0.01$) compared to FRP. The heart rate with FRP was on the average 72 beats/min at rest and increased to 83 beats/min during work. This difference in average heart rate with FRP was caused by interference of normal, conducted sinus beats which was observed in three patients at rest and five during exercise. Because of the moderate increase in heart rate, the increase of cardiac output during exercise with FRP was mainly caused by a marked increase in stroke volume from 72 ml at rest to 104 ml during work. With ATP on the other hand, the increase of cardiac output during exercise was brought about primarily by an increase of heart rate from 83 beats/min at rest to 126 beats/min, and only to a minor degree by an increase of stroke volume, from 60 ml to 81 ml.

The filling pressure of the left ventricle as reflected by the mean pulmonary artery wedge (PCV) pressure or diastolic pulmonary artery pressure was on the average significantly lower ($P < 0.01$) with ATP than with FRP at rest, 9.2 and 12.5 mm Hg respectively. During work, there was no significant difference in filling pressures ($P > 0.8$) (23.9 mm Hg with ATP and 24.3 with FRP). As mentioned above, interference between fixed pacemaker induced rhythm and conducted sinus rhythm occurred in five patients during exercise. When these five patients were excluded, the difference in cardiac output during work with ATP compared to FRP increased to 27 % and the PCV pressure was significantly lower with ATP on the 5 % level.

No patients in the study had a history of angina pectoris. In spite of that, two patients got angina pectoris during exercise when on ATP whereas on FRP they could perform the same work load without any discomfort. The signs of coronary insufficiency were obviously caused by increased myocardial oxygen consumption, when the heart was working at a higher rate and transporting more blood.

IV INDICATIONS FOR TREATMENT WITH ATRIAL TRIGGERED PACEMAKER

This presentation of the indications and contraindications for treatment with ATP is mainly based on findings presented in paper V and on subjective observations reported by patients treated with both fixed rate and atrial triggered pacemakers.

Most patients with a normal atrial activity and without coronary insufficiency feel better with ATP than with FRP. The cardiac output is higher and the ventricular filling pressure lower in most cases. Lack of coordination between atrial and ventricular systole, as with FRP results in a cyclic variation of the arterial pressure, which may be pronounced in patients with a low compliance of the ventricular wall, such as in aortic and pulmonary stenosis and in hypertrophic cardiomyopathies. In these patients the varying systemic blood pressure on FRP causes symptoms which vanish on ATP. The most important advantage with ATP is the ability to increase the ventricular rate during work. Young, and otherwise healthy patients, who are used to being physically active and therefore need a high oxygen transport capacity should be offered an ATP. In very old patients, the physical activity is generally not limited by the circulatory capacity and then an FRP is satisfactory.

Patients who have an unstable AV-block, i.e. are changing between sinus rhythm and complete block, should have some type of triggered unit, as FRP in these patients gives rise to competition between sinus rhythm and pacemaker induced ventricular activity. This interference rhythm induces a risk for sudden death, because of the well known fact that an impulse from a fixed rate pacemaker falling in the vulnerable period (in the T wave) of the Ecg of a spontaneous beat can under certain but rare circumstances provoke a ventricular tachyarrhythmia, which can result in ventricular fibrillation. The risk for this complication must, however, be small in most patients under normal conditions, as it was possible in the beginning of the pacemaker era to treat all patients, even those who most of the time were in sinus rhythm with fixed rate pacemakers, without incurring a high mortality. In materials from this time, varying incidents of sudden death have been reported. Sowton reported 1965 a five times higher incidence of sudden death in patients with competitive rhythm compared to those who followed the pacemaker without interference (37). Edhag, on the other hand, did not find a definite increase in death rate among patients with competition (13). Certainly these differences can be caused by differences in the materials. The fibrillation threshold could also be temporarily lowered in a patient, because of myocardial damage due to anoxia or electrolyte disturbance. This view is supported by observations made by Edhag on infarction patients in a cardiac care unit (14). He noticed that patients with coronary insufficiency and interference between pacemaker induced rhythm and sinus rhythm, had a higher incidence of ventricular tachyarrhythmias than patients with competition rhythm, but without coronary insufficiency. In a study of the vulnerable period Weltı et al. (40) have reported two patients with recent myocardial infarctions and AV-block III, in whom each pacemaker impulse falling in the vulnerable period gave rise to ventricular fibrillation. The amplitude of the impulses in these two patients was just above the threshold for stimulation.

An argument for treating patients, who part of the time have conducted sinus rhythm, with ATP is the possibility that they get a retrograde activation of the atria on FRP. The resulting rhythm has a very bad haemodynamic effect, as examplified by a typical patient reported in papers IV and V. This patient had attacks of AV-block III, but was most of the time in sinus rhythm. The cardiac output at sinus rhythm was 6.1 l/min (HR 78 beats/min). When connected to the asynchronous pacemaker (HR 88 beats/min) the cardiac output decreased to 4.3 l/min, and the filling pressures of right and left ventricles rose markedly. When an atrial synchronous pacemaker was connected (HR 78 beats/min) the cardiac output increased again to 6.3 l/min and the filling pressures decreased. The explanation of the very marked decrease in cardiac output and stroke volume with FRP was retrograde activation of the atria, which, instead of contributing to the filling of the ventricles in end-diastole, regularly contracted against closed atrio-ventricular valves and emptied in a retrograde direction into the big veins. This retrograde pumping of the atria is also often noticed by the patients as an unpleasant sensation in the chest.

For patients who most of the time have conducted sinus rhythm, some type of ventricular triggered (R wave inhibited or R wave synchronous) pacemaker is the type of choice. Some of these patients, however, use to get complete block regularly when performing heavier exercise. It is obvious that this implies an indication for the somewhat more complicated treatment with ATP. In a discussion about patients with unstable complete AV-block, it has to be pointed out that this diagnosis cannot be made until the pacemaker treatment has started. It is a common observation that in quite a few patients, who before treatment have had a stable complete AV-block III for a long time, the heart starts to interfere with conducted sinus rhythm, when a pacemaker has been connected for some time. Because of this, a patient who is about to get his first pacemaker implanted should either have some type of triggered unit or should first be treated for some time with an external pacemaker.

V CONTRAINDICATIONS FOR TREATMENT WITH ATRIAL TRIGGERED PACEMAKER

Patients who have or have had atrial arrhythmias, such as atrial fibrillation or flutter sinus bradycardia or sinus arrest, should not be treated with ATP If however a patient treated with ATP gets atrial flutter or fibrillation this does not usually disturb him too much. The electrical amplitude led off from fibrillating atria is usually too low to trigger the pacemaker which because of this gives impulses at its basic rate of around 50 impulses/min. The amplitude of atrial flutter usually is high enough to trigger the pacemaker but as the resulting impulse rate due to the construction of the device is generally around 100—130 impulses/min, the patient only feels a minor discomfort.

Coronary insufficiency is a contraindication against treatment with ATP The physical activity in patients with ischemic heart disease is limited by the chest pain which will occur at a lower work load at ATP because of the higher heart rate and myocardial oxygen consumption as compared with FRP It is also a common clinical observation that these patients have a better exercise tolerance with FRP than with ATP

A relative contraindication for treatment with ATP can be that the patient has an occupation, in which he is exposed to strong electromagnetic interference, which could trigger the rather sensitive pacemaker Such external electromagnetic disturbance of the pacemaker's activity does not imply a life risk, but is unpleasant for the patient.

Conclusions

Indications for ATP

1 AV-block III in physically active patients with normal atrial activity

2 Patients with retrograde activation of the atria on FRP

3 Patients in sinus rhythm at rest who get AV block III during exercise

4 Some patients with low compliant myocardia, such as aortic and pulmonary stenosis and hypertrophic cardiomyopathies, where the atrial contraction is essential for the necessary filling pressure

Contraindications for ATP

1 Atrial arrhythmias such as atrial flutter and fibrillation, sinus bradycardia, sinus arrest.

2 Ischemic heart disease.

3 Occupational risks of frequent electromagnetic disturbance of the pacemaker

General summary

The present investigations were planned to deal with the influence of heart rate on central haemodynamics, left ventricular performance and peripheral blood flow in patients with acquired complete heart block. Pacing of the heart was induced in the right ventricle. Heart rate was varied either artificially by alteration of impulse frequency of a fixed rate pacemaker or in the case of an atrial triggered pacemaker by the spontaneous sinus rate of the patient. Studies were performed both at rest and during work, in steady state conditions. These measurements were supplemented by studies of the effect of sudden changes in heart rate on peripheral blood flow, blood pressure and left ventricular performance.

Fifty-three patients suffering from AV-block III were studied. The mean age of the patients is lower than that generally observed in pacemaker patients.

In the course of the work, a new pacemaker was developed which allowed the ventricular rate to be controlled by the atrial electrical activity with normal time relation between atrial and ventricular contraction. The ventricles were paced by means of a transvenously introduced electrode, the tip of which was positioned in the apical region of the right ventricle. The atrial impulse was sensed with an electrode of the same design positioned by means of mediastinoscopy close to the atrial wall, where a P wave amplitude of more than 1 mV could be obtained.

Exercise was performed in supine on a bicycle ergometer. Central haemodynamics were studied by means of heart catheterization with measurement of pressures and flow. Regional blood flow was measured with venous occlusion plethysmography and left ventricular volume was calculated from cine films, which were taken after a right atrial injection of contrast medium.

The effect of varying the rate of right ventricular pacing was first studied at rest in 22 patients. At idioventricular rhythm with a mean heart rate of 34 beats/min, the circulation was markedly hypokinetic, and increasing the rate by means of the fixed rate pacemaker resulted in an increase of cardiac output, without a change of oxygen uptake shifting the kinetics of the circulation to a more normal state. Further increases in heart rate, up to rates between 152 and 166 beats/min, resulted in only minor changes in cardiac output. Stroke volume at rest was close to its maximum at idioventricular rhythm and decreased when artificial pacing was initiated. Within the range of pacing rates, where cardiac output remained more or less constant, stroke volume varied inversely with heart rate. The filling pressure of the right ventricle, measured as right atrial mean or right ventricular end-diastolic pressure, varied with stroke volume, i.e. decreased with increasing heart rate.

The effect of varying rate during exercise only dealt with studies where the artificial pacemaker was used. With exercise at a fixed ventricular rate, the cardiac output increased in all patients, but was usually lower than normal in relation to oxygen uptake. Consequently the stroke volume increased in proportion to the work load. At a constant work load, the stroke volume and right ventricular filling pressure decreased with increasing ventricular rates, and the cardiac output increased only slightly. The latter study was performed at ventricular rates between 55 and 110 beats/min.

It is concluded that above a certain lower heart rate level, cardiac output is determined by the metabolic activity of the-body and not by heart rate per se. The fact that the cardiac output is constant implies that stroke volume varies inversely with heart rate. The filling pressures during exercise

were higher than at rest, but decreased with increasing rate.

With the aim to study the effect of sudden changes of heart rate, forearm blood flow was recorded with venous occlusion plethysmography in 6 patients at rest in supine body position. Heart rate was changed over a wide range (30—150 beats/min). The plethysmography curve allowed beat-to-beat forearm pulse volume changes to be determined. Arterial blood pressure was measured via a catheter in the brachial artery of the opposite arm.

Sudden increases or decreases in heart rate did not affect forearm blood flow to any significant extent. Forearm pulse volume and arterial pulse pressure varied inversely with heart rate. These changes in pulse volume and pulse pressure occurred from one beat to the next.

Assuming that variations in forearm blood flow reflected variations in cardiac output, and that changes in pulse volume and arterial pulse pressure reflected changes in stroke volume of the heart, it was concluded that sudden changes in heart rate do not change cardiac output to any significant extent, and that stroke volume decreases with increasing heart rate. These changes occur from one beat to the next when heart rate is changed stepwise.

The cineangiographic volume studies were performed at rest in supine position. Heart rate was changed step-wise by means of an external pacemaker while contrast medium was passing through the left ventricle, thus allowing a beat-to-beat study of the volume changes. A sudden increase in rate resulted in a decrease in stroke volume from one beat to the next. This decrease was effectuated by a decrease in the end-diastolic volume of the left ventricle while the end-systolic volume remained unchanged. A transient increase of the calculated cardiac output was observed when heart rate was increased within certain limits. The magnitude of this increase in cardiac output was related to whether the control level of the heart rate was subnormal or not. A decrease in heart rate resulted in an increase of the stroke volume and of the end-diastolic volume of the heart.

The importance of a properly timed atrial systole for central haemodynamics was studied in patients at rest and during exercise, paced alternatively with atrial triggered and with fixed pacing, at approximately the same rate as that obtained with atrial triggered pacemaking. With this experimental design, all other variables were assumed to remain unchanged.

At the same oxygen uptake at rest and during exercise, cardiac output was on the average 0.75 l/min higher with atrial triggered pacing. At atrial triggered pacing this increase was effectuated by an increase in stroke volume, which was larger at rest than during exercise.

To evaluate the importance of an increasing heart rate for central haemodynamics in patients with complete AV-block, studies were made at rest and during exercise at the same load with atrial triggered pacing and fixed pacing at a rate of about 70 impulses/min. With this experimental design, the combined effect of the synchronized atrial contraction and the spontaneous increase in rate was compared to fixed rate pacing. With fixed rate pacing, the circulation was slightly hypokinetic at rest and more so during exercise. The increase in cardiac output during work was accomplished by large increases in stroke volume and by increases in ventricular filling pressure. With atrial triggered pacing at the same oxygen uptake the circulation was less hypokinetic and the increase of stroke volume less marked. In spite of the higher cardiac output, ventricular filling pressure was the same or somewhat lower. Thus, with atrial triggered pacing, a larger cardiac output may be obtained with a lower ventricular filling pressure.

GENERAL CONCLUSIONS

At fixed rate ventricular pacing metabolic activity is the main determinant of cardiac output above a certain lower limit of heart rate both at rest and during exercise.

Stroke volume and filling pressures vary inversely with heart rate above this critical rate, and cardiac output remains relatively constant.

Sudden changes in heart rate are met by changes

in stroke volume from one beat to the next, decreasing with increasing heart rate.

This decrease in stroke volume is effectuated by a decrease in end-diastolic ventricular volume, while the end systolic volume remains unchanged.

Normal timing of atrial and ventricular contraction results in measurable increases in cardiac output compared to asynchronous activity

Atrial triggered pacing, when applicable, is a superior treatment of complete AV-block, allowing a more normokinetic circulation during exercise compared to fixed rate pacing.

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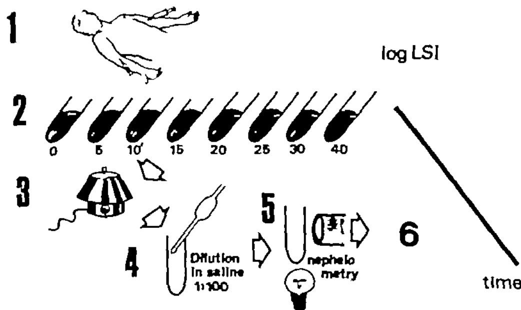
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Studies on an Intravenous Fat Tolerance Test

Methodological, Experimental and Clinical Experiences with Intralipid®

By Stephan Rössner



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II S. Rössner, J. Boberg, L.A. Carlson, Ulla Freyschuss and B.W. Lassers. Comparison between fractional turnover rate of endogenous plasma triglycerides and of Intralipid (Intravenous fat tolerance test) in man. Europ J clin Invest. 4 109 1974

III L.A. Carlson, L. Kaijser, S. Rössner and M.L. Wahlqvist. Myocardial metabolism of exogenous plasma triglycerides in resting man. Acta med scand 193 233 1973

IV Ulla Freyschuss, D. Hallberg, L. Johansson and S. Rössner. Removal of exogenous plasma triglycerides in splanchnic viscera in man during anesthesia. Accepted for publication in Acta med scand

V L. Kaijser and S. Rössner. Extraction of exogenous triglycerides in human forearm muscle and subcutaneous tissue. Accepted for publication in Acta med scand

VI. S. Rössner, U. Larsson-Cohn, L.A. Carlson and J. Boberg. Effects of an oral contraceptive agent on plasma lipids, plasma lipoproteins, the intravenous fat tolerance and postheparin lipoprotein lipase activity. Acta med scand 190 301 1971

VII. S. Rössner and U. Rosenqvist. Serum lipoproteins and the intravenous fat tolerance test in hypothyroid patients before and during substitution therapy. Accepted for publication in Atherosclerosis

These publications will be referred to in the text by their Roman numerals

CONTENTS

INTRODUCTION

Elevated concentrations of blood lipids are now commonly accepted as risk factors for the development of atherosclerotic diseases. This holds true not only for hypercholesterolaemia but has also been found to be valid for hypertriglyceridaemia (1, 2, 3). The rapid development within the field of atherosclerosis research has broadened our present knowledge about the relation of elevated blood lipids to atherosclerotic diseases since the introduction of the lipoprotein concept (4). These lipoproteins have been classified by various methodological approaches such as ultracentrifugation, electrophoresis or nephelometry (5, 6, 7, 8). One of the most useful techniques to separate lipoproteins is to use the preparative ultracentrifuge. With this technique three major lipoprotein classes are isolated: Very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL), and in these fractions TG and cholesterol can subsequently be determined (8).

Following the absorption of dietary fat chylomicrons are formed. These particles contain mainly TG and are rapidly removed from the circulation. After an overnight fast chylomicrons are normally not present in the blood. VLDL are mainly synthesised in the liver. In the fasting state most of the circulating TG are carried in the VLDL, which also have a rapid turnover rate. In VLDL a small part of the circulating plasma cholesterol is also found. However, the main part of the circulating cholesterol is transported in the LDL fraction which generally carries only a small proportion of TG. The HDL fraction contains mostly cholesterol and phospholipids and only little TG. This fraction may however play an important role in the transport of VLDL TG in the circulation. The function of the plasma lipoproteins has recently been reviewed by Robinson (9).

Hypertriglyceridaemia is the result either of an increased secretion of TG into the circulation or of an impaired removal. These processes may also be combined. During recent years evidence has accumulated that hypertriglyceridaemia is caused by a removal defect rather than by an increased secretion of TG into the circulation (10). It is therefore natural that various methods have been developed to study the elimination of plasma TG from the blood stream.

The first methods used for this purpose were oral fat loads of different kinds (11). In these tolerance tests fats of different kinds were administered orally. After a standardised fatty meal the turbidity of blood plasma was used as a measurement of the TG concentration. Tracers such as labelled fats, vitamin A etc were also administered and the concentration of these tracers in blood plasma was determined by isotopic TG analysis, vitamin A measurements etc.

Oral tests were found to have disadvantages, because it was difficult to evaluate the time course for exogenous plasma TG removal from the blood stream for the following reasons. The blood levels during such tests depend not only on the rate of disappearance of exogenous TG from the blood stream but also on various absorption processes in the gastro-intestinal tract, the formation rate of chylomicrons in the gastro-intestinal tract, the influx into the circulation, the possible appearance of "endogenous" TG in the circulation after the removal of chylomicrons etc. The shape of such TG elimination curves may thus depend on several complex processes and be difficult to interpret.

In the following discussion "exogenous lipids" are used as a term for lipids in chylomicrons entering the blood stream from the thoracic duct during absorption of dietary fat or lipids in artificial fat emulsions given intravenously. If these lipids reappear in plasma after once having left the blood stream, they are called "endogenous lipids". Endogenous plasma TG may also be newly synthesized.

Several methods have been introduced to bypass the influence of the gastrointestinal tract on TG resorption and chylomicron formation. A physiological way to determine the removal of exogenous TG from the blood stream is to study the elimination of intravenously injected chylomicrons. Although such studies have been carried out in both experimental animals and in

man (12 13) It is not possible for several prac tical reasons to use such a method as a clinical routine test Interest has therefore been focused on fat emulsions that can serve as substitute for chylomicrons. For such a test to be valid the fat emulsion must serve as a tracer of chylomicron metabolism. Fat emulsions of different composition or with isotopically labelled TG have therefore frequently been used (14 15) Direct uptake of chylomicron TG by liver skeletal muscle and adipose tissue as well as lipolysis have been mechanisms believed to result in chylomicron TG clearance from blood (9) Many artificial fat emulsions however seem to be taken up by the reticuloendothelial system to such an extent that they cannot be regarded as tracers of chylomicron metabolism (16)

The fat emulsion Intralipid® (Vitrum AB Stockholm, Sweden) has been found to have excellent clinical tolerance (17) Intralipid® re sembles chylomicrons in several aspects They have similar enzyme kinetic behaviour as substrate *in vitro* with post-heparin lipase and the kinetics for the elimination of Intralipid® and chylomicrons are identical in dog and in man (12 18 19) There are thus several reasons why Intralipid® may be a valid tracer for chylomicron metabolism.

In 1963 Carlson and Hallberg demonstrated that after an intravenous single dose injection of Intralipid® the disappearance of the emul sion from the blood stream could be charac terised by two rate constants (12) At high Intralipid® concentrations a maximal removal and zero order reaction rate (k_1 μmol/min) was found and below a critical concentration the removal rate was of the first order (k_2 %/min) Based on these findings Boberg Carlson and Hallberg introduced an intravenous fat tole rance test (IVFTT) 1969 using the Intralipid® emulsion and determining k_2 (0) k_1 and k_2 were not related to each other nor were k_1 and the plasma TG concentration in this study How ever k_2 was found to correlate with the plasma TG concentration in a reciprocal way so that subjects with high k_2 values had low plasma TG concentrations and *vice versa*.

The negative relationship between the IVFTT and the serum TG concentration has been analysed in several clinical studies (21 22 23 24 25) However in order to obtain data on k_2 characteristics also from healthy subjects with normal serum lipid levels and to compare these with the correspon ding values obtained from a group of indivi duals with asymptomatic hyperlipoproteinae mia, a population study has been carried out (26) This study was performed on apparently healthy subjects attending a health control cen tre The hyperlipoproteinaemic subjects parti cipating in the investigation were active professional people who at the health control centre incidentally were found to have elevated se rum lipids. They comprised about the upper 3 per cent of the population analysed and were referred to the Lipid Unit Karolinska Hospital. At the Lipid Unit a detailed medical history was taken and an extensive clinical and laboratory examination was carried out Sub jects who had a history of atherosclerotic mani festations or of secondary hyperlipoproteinae mia or were treated with drugs expected to affect lipid metabolism were excluded Some of the subjects classified as hyperlipoproteinaemic during the screening procedure had "normal" lipid values when investigated at the Lipid Unit These subjects were also included in the study and referred to as "normals" Matched controls were selected from the same health control centre The controls were of the same age and sex as the corresponding hyperlipoproteinaemic subjects, but had a serum TG concentration below 2 mmol/l and a serum cholesterol con centration below 270 mg/100 ml on at least three occasions. Fasting serum lipids and the IVFTT k_2 value were determined as described (27 28 study I) The sera of the subjects were typed according to a WHO report (29). Clinical data and results from this study are summarised in Table I and the relationships between k_2 and serum TG in the different groups are given in Fig 1

In earlier studies on the negative relationship between k_2 and fasting TG concentration there has been an impression that this relation rather

Table I Clinical data total serum TG and cholesterol on the IVFTT occasion in control and hyperlipoproteinaemic subjects (ref 26)
Mean±SEM are given.

		n	Age years	TG mmol/l	Chol mg/100 ml	Weight/Height index kg/cm 100	IVFTT k_2%/min	IVFTT k_2 range
Control subjects		M 45	48±2	1.01±0.05	?22±5	0.96±0.02	6.09±0 41	2.21 19.88
		F 43	53±1	1.03±0.04	234±6	0.94±0.01	7 15±0.33	4.21 11.22
Hyper lipopro-teinaemic subjects	"Normals"							
		M 27	48±2	1.91±0.22	263±8	0.99±0.03	3 82±0.31	1 74- 8.71
		F 24	54±2	1 75±0 13	302±8	0.99±0.03	5.05±0 45	2.11 11.32
	Type IIa							
		M 27	49±2	1.68±0.09	350±10	0.98±0.0?	4.30±0.31	2 41-8 16
		F 37	56±1	1 79±0 10	351±9	0.98±0.02	5.21±0.31	2.26-9.34
	Type IIb							
		M 15	52±2	2.57±0.27	340±12	1.03±0.02	2.89±0 15	2.05-3.88
		F 12	56±2	3.02±0.24	349±14	1.02±0.04	3.58±0.25	1.91-4 73
	Type III							
		M 12	48±3	3 73±0 79	296±17	0.99±0.03	3 43±0 41	1.83-6.42
		F 6	55±3	3.21±0 13	335±31	1.08±0.09	4 16±0.52	2.35-5.88
	Type IV							
		M 94	48±1	4.20±0.28	274±5	1.08±0.01	? 78±0.09	1.51-4.97
		F 20	55±3	3 40±0.59	308±15	1.08±0.03	3.86±0.36	1.37-6.45
	Type V							
		M 3	46±7	18 1±11.20	353±87	1.06±0.09	2.02±0.63	0.84-2.98
		F						

Table II Comparison between regression equations for male and female subjects with hyperlipoproteinaemia The r-value and Student's t-value are given for linear regression and double logarithmic regression.

	Linear regression		Double logarithmic regression	
	Males	Females	Males	Females
	-0.29	-0 46	-0.53	-0.55
t	3 73	-4.83	7 74	-6 19

was of a hyperbolic than a linear type (20 22 23 24) This has been tested in the present material and the results are given in Table II The comparison between linear and double logarithmic regression revealed that higher r-values and Student s t values were found for the logarithmic regression supporting the concept of a hyperbolic relation between k_2 and plasma TG concentration In the following presentations the relationships between k_2 and TG have therefore been analysed in double logarithmic plots

The data in Table I represent very wide ranges of k_2 values and serum TG concentrations. Fig 1 demonstrates that the mean values of these different groups together formed a negative relation between k_2 and serum TG also when the extreme values obtained from males with a type V pattern or male and female controls were included The groups indicated in Fig. 1 represent different and distinct lipoprotypes by definition but still formed a continuous relation. All female groups, however seem to be found to the right of the corresponding male group Furthermore it is also possible that male and female subjects with a type III pattern were found towards the right part of the relation curve Although the mean value for k_2 was lower in subjects with elevated serum TG concentrations there was a considerable overlap of hyperlipoproteinaemic and control subjects Several subjects with hypertriglyceridaemia had higher k values than individual controls with low TG values This finding might illustrate that the lipoprotein typing system according to the WHO classification is a static way to define hyperlipoproteinaemias and does not take into account more dynamic aspects of the lipoprotein metabolism There is now accumulating evidence that hyperlipoproteinaemia is a much more heterogeneous condition than was previously thought In a recent paper Lees (30) has underlined the importance that data on plasma con n tion of lipids are combined with kinet p ameters of lipoprotein metabolism in orde t enable an a urate definition of the phen pes that reflect the inherited biochemical d ts underlying the primary hyperlipoproteinaemias The findings of the population study demonstrated that although a significant correlation between k_2 and serum TG was found in a large population there was a considerable overlap of k_2 values between subjects with elevated serum TG concentrations and controls These observations prompted the present series of investigations with the general aim to study whether the IVFTT k_2 value could provide additional information about the dynamic aspects of lipoprotein metabolism and thus be of value in a more integrated system to classify hyperlipoproteinaemia

Boberg Carlson and Hallberg suggested that the IVFTT might be of clinical value in several ways As a method to study physiological and patho-physiological aspects of TG metabolism as a tool to evaluate the effects of treatment of hyperlipoproteinaemia and as a method that could possibly discover subjects prone to hypertriglyceridaemia and coronary heart disease

If this would hold true the IVFTT would resemble the intravenous glucose tolerance test (IVGTT) in several aspects as a tool for metabolic studies Both methods determine the elimination course of a substance given intravenously as a single dose injection

When the IVFTT was introduced it was unknown to what extent the test could be applied in the examples mentioned above Furthermore several methodological problems remained unsolved In the first report on the IVFTT exogenous TG were first separated from endogenous TG by a PVP gradient method (31) followed by determination of the TG concentration in the isolated fractions. This method is a very tedious procedure and not suitable to apply if the IVFTT should serve as a clinical routine method

AIM OF THE PRESENT STUDY

1 To develop a clinical routine method to perform the intravenous fat tolerance test (IVFTT) in man and experimental animals and to develop a laboratory method to analyse the disappearance of Intralipid® from the blood stream.

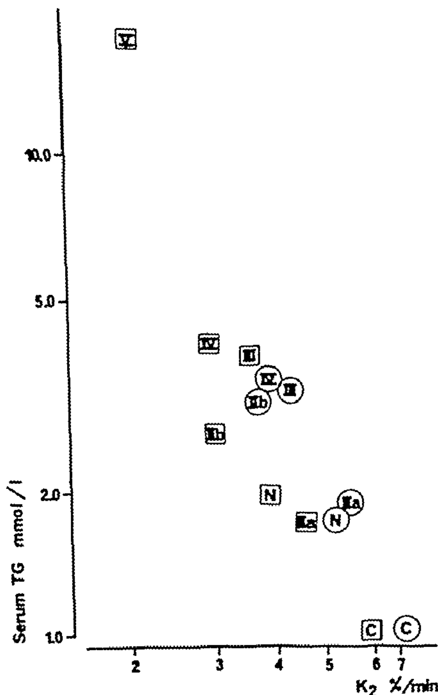


Fig 1 Relationship between k_2 and serum TG in groups of subjects with varying concentrations of lipoproteins (Table I) C = control subjects, N = subjects initially classified as hyperlipoproteinaemics but with sera typed as normal on subsequent analysis. Roman figures indicate type of hyperlipoproteinaemia Males in open squares, females in open circles. Logarithmic scales.

2. To investigate whether the IVFTT could reflect fractional turnover rate also of endogenous plasma TG
3. To investigate in which tissues the Intralipid® emulsion is removed
4. To apply the IVFTT in the study of individuals whose plasma lipoprotein concentrations were changed either by disease or by treatment with drugs and to study the relationships between k_2 and lipoproteins after such manipulations in the same individuals

SUBJECTS

In the methodological study (I) male and female volunteers and patients with various types of hyperlipoproteinaemia were studied. In study II male subjects with "normal" or elevated plasma TG concentrations according to the Stockholm Prospective Study (3?) were investigated. In studies III and V male volunteers with previous history or symptoms suggestive of cardiovascular or metabolic disorder were studied. In study IV patients, admitted to the department of surgery, Karolinska Hospital for cholecystectomy were studied. These patients were all free of symptoms suggestive of acute cardiovascular or metabolic disease and were not on any medication expected to affect lipid metabolism. In all patients an informed consent to the study according to the Helsinki declaration was obtained. No complications were observed and the recovery after the operation was uneventful in all cases. In study VI 1 healthy female premenopausal subjects without any history or symptoms indicating a metabolic disorder were studied. Study VII comprised nine female hypothyroid patients.

METHODS

The intravenous fat tolerance test (I)

The IVFTT was always carried out after an overnight fast and with the subjects in supine position. An indwelling [illegible]theter was inserted into an antebrachial vein [illegible] nd was used both for Intralipid® injection and blood sampling. The following routine method for the intravenous fat tolerance test was developed. A single dose of the fat emulsion (1 ml 10 % Intralipid®/kg body weight) was given intravenously. Blood samples were drawn every five minutes for forty minutes and the light scattering index of plasma diluted 1 100 in saline was determined by means of the Thorp micronephelometer (33). Capillary samples could be used as well, which makes it possible to apply the method *when there are difficulties* to use a permanent intravenous cannula such as in patients who do not have veins that are easily accessible in children or in laboratory animals. In earlier studies on the IVFTT exogenous TG were separated from endogenous TG by a PVP-gradient technique (31). This method is tedious and so a method using the Thorp micronephelometer was developed. Comparisons between nephelometry and PVP-gradient determinations have previously been shown to agree excellently (34). Since the start of the present study more than ?100 IVFTTs have been carried out. No adverse reactions of any kind have been observed.

Comparisons between exogenous and endogenous plasma TG fractional turnover rates (II)

The fractional removal rate determined by the IVFTT was compared with the fractional turnover rate of endogenous TG in paper II where the plasma TG turnover had been quantified (35). In these studies the endogenous plasma TG turnover was studied in two ways based on either a chemical secretion method or a "plasma clearance method". The first method determines the splanchnic secretion rate of unlabelled plasma TG calculated as the concentration d[illegible]fference between artery and the hepatic vein multiplied by estimated hepatic plasma flow. The se[illegible]ond method estimates the amount of T[illegible] [illegible]emoved from the circulation over a [illegible]ert[illegible]n pe[illegible]od of t[illegible]me by means of labelled TG. The [illegible]alue f[illegible]r the [illegible]fra[illegible]tional turnover rate" of end[illegible]ge[illegible]n[illegible]us plasma TG was [illegible]alculated by dividing the [illegible]lues [illegible] plasma TG turnover rate by the pl[illegible]sma [illegible]

Procedures in study III IV and V

In three studies (III, IV V) the removal of Intralipid® in the following tissues was studied The myocardium (III) the splanchnic region and the liver (IV) and the skeletal muscles and subcutaneous tissue of the forearm (V) The procedures which were used in these studies are described in detail in the individual papers. In this section they are only briefly summarised

All subjects were studied in the morning after an overnight fast In study III the concentration differences of Intralipid® across the myocardium were determined by means of catheters in a brachial artery and in the coronary sinus. In study IV a brachial artery the hepatic vein and the portal vein were catheterised during anesthesia and the concentration differences of the fat emulsion were determined across the splanchnic region and across the liver In study V a brachial artery a deep and a superficial antebrachial vein were catheterised and concentration differences were studied across the fore arm skeletal muscle and across the forearm subcutaneous tissue

In these three studies blood samples were drawn simultaneously from the catheters used for sampling Dry syringes were always used for the samples for nephelometry and blood was taken into tubes with lyophilised heparin No heparin was used to keep catheters patent but only slow saline drips or intermittent flushes with saline

Plasma water shifts

As it was to be expected that any existing concentration differences would be small, it was essential that any systematic shift of plasma water which might occur during the passage of the blood across the systems studied could be detected To establish if such shifts of water did occur ^{125}I-albumin was used as a tracer for plasma albumin It was given to the subjects studied in III IV and V one to four days before the study In replicates of samples drawn from the respective catheters the ^{125}I radioactivity was subsequently determined With this method it should be possible to detect shifts in the order of 1% of the arterial concentration.

Clinical studies

In studies VI and VII the lipoprotein metabolism was studied in patients where the plasma lipoprotein concentration was increased In study VI healthy female subjects without clinical symptoms of metabolic disease were studied before and during treatment with an estrogen containing oral contraceptive In study VII hypothyroid female patients were studied before and during substitution with thyroid hormone In both these studies plasma lipids lipoproteins and the IVFTT were determined before and during drug treatment.

DISCUSSION OF METHODS

In studies III IV and V the Intralipid® uptake in various tissues was studied by means of nephelometry determinations of exogenous TG In these studies the assumption has been made that nephelometric concentration differences across a vascular bed indicate a removal of Intralipid® particles and that no change in particle size appears. This assumption may be valid for the following reason In study III the nephelometric arterio-coronary sinus differences were compared with a chemical method where plasma TG was determined by measuring total plasma glyceride-glycerol by an AutoAnalyzer technique (77). These values were corrected for free glycerol (35). The grand means for the two methods in study III were for the chemical method 54±24 µmol/l nephelometric method 46±27 µmol/l. In studies IV and V a comparison was made between the nephelometric determination of Intralipid® TG uptake and a chemical method where lipids were extracted by means of a chloroform-methanol Folch extract on which triplicates were analysed ten times each (7 36). This chemical determination gives a very high precision In the subjects studied, a close correlation was found between the nephelometric data and the values from the chemical method The grand means for the two methods in studies IV + V were for the chemical method 66±18 µmol/l and for the nephelometric method 67±8 µmol/l. These results taken together suggest that nephelometry is a

valid method to determine concentration differences of Intralipid® over the vascular beds studied in papers III IV and V

RESULTS AND COMMENTS

Methodological studies on the IVFTT (I)

In study I it was shown that a linear standard curve was obtained when Intralipid® was diluted This curve included the origin This finding made it possible to use a standard curve for the conversion of plasma light scattering index (LSI) to μmol Intralipid® TG/l in studies III IV and V When 1 ml 10% Intralipid® per kg body weight was given first order kinetics were generally demonstrated for the removal of the fat emulsion The slope of the regression line was determined by the method of the least squares and the equation log Y = b X + a was calculated The disappearance rate k_2 of the fat emulsion was expressed as $-b \pm s_b$ where s_b is the standard error of b A typical example of the data obtained from an IVFTT is given in Fig 2 The slopes by which k_2 was determined were generally linear No upward slope towards the end of the curve was found which makes it unlikely that circulating "secondary particles" (37) have interfered with the nephelometric readings

In study I it was demonstrated that the plasma samples could be stored at least two weeks before reading without affecting the k_2 value or its methodological error This finding is of practical value in a laboratory routine k_2 was highly reproducible in the same individual when repeated consecutive days Further studies have demonstrated that a similar good reproducibility is found when subjects are retested with an interval from one week up to six months (Fig. 3) (38).

Comparison between IVFTT and endogenous plasma TG fractional turnover rates (II)

In 32 male subjects with wide ranges of the plasma TG concentration the fractional turnover rate of endogenous TG was determined and the IVFTT was carried out A significant correlation between fractional turnover rate of endogenous TG as determined by these methods and the IVFTT was obtained The r-value for the relationship between the "chemical secretion method" and the IVFTT was 0 60 ($p<0.01$) and the r-value for the relationship between the "clearance method" and IVFTT was 0 71 ($p<0.001$). Because of methodological reasons the error for the determination of endogenous plasma TG turnover rate is quite high around 40% A statistical analysis of the relationships revealed that still higher r-values would have been observed if the methodological error of the endogenous plasma TG turnover study had been lower

In this study a significant negative correlation was found between the fractional removal rates of both endogenous and exogenous plasma TG and VLDL–TG (r = –.65 – –0 78)

Intralipid® removal sites (III IV V)

In studies III IV and V the arterio-venous concentration differences of Intralipid® TG particles were studied by means of nephelometry across various tissues. In study III it was demonstrated that about 6% of the arterial concentration of Intralipid® TG was removed during the passage through the myocardial tissue In study IV the arterio-portal concentration difference of Intralipid® averaged about 5% of the arterial concentration. There was however no significant portal-hepatic venous concentration difference of Intralipid® TG across the liver In study V the corresponding figures for removal of Intralipid® TG particles were about 10% in the forearm skeletal muscles and 6% in the forearm subcutaneous tissues

Blood plasma flow during the Intralipid® infusion was estimated in study IV This study demonstrated that the Intralipid® infusion did not change the estimated splanchnic plasma flow Under the assumption that the plasma flow was unchanged in the other tissues during a constant infusion of Intralipid® it is possible to make a rough estimate of the role of the various tissues in the removal of Intralipid® from the circulation (Table III) The figures for plasma flow have been taken from Wade *et al* (39) and the heamatocrit has been assumed to

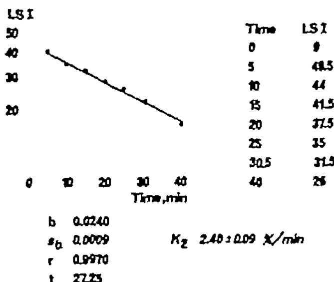


Fig. 2. Disappearance curve of Intralipid® during an intravenous f t tolerance test. After subtraction of a blank taken at zero time the light scattering indices are plotted against time in a semilogarithmic plot By means of a standard computer program the slope of the curve is determined by the method of least squares. The disappearance rate k_2 of the emulsion is expressed as $-b \cdot ln_b$ and given as the percentage of the emulsion cleared from plasma per minute *The r-value and Student s t are also given*

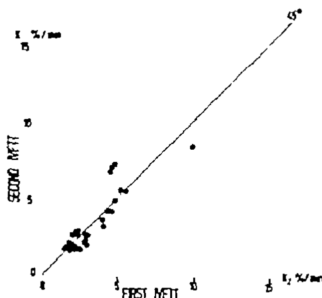


Fig 3 Relationship between k_2 values calculated from IVFTTs carried out with time intervals between one week and six months. The individuals studied were not on medications or diets e pected t affect the k_2 values or serum TG concentrations and had a wide range of fasting serum TG concentrations The IVFTT was carried out in identical ways on both study occasions. The line of identity is indicated in the plot.

average 40%. In the calculation presented in Table III it has further been assumed that the subjects studied are resting supine as muscular exercise possibly can influence the removal of Intralipid® TG particles (Study V). In study IV the patients studied were anesthetised whereas in studies III and V they were conscious and not premedicated. The possible Intralipid® removal differences induced by the anesthesia given to patients in study IV have therefore not been taken into account in the calculation either. Under these assumptions and bearing in mind that the data in Table III are combined from different studies and calculated in each study separately it seems possible to account for the removal of Intralipid® in various tissues. Table III demonstrates that almost 50% of the infused Intralipid® was removed in skeletal muscle whereas the splanchnic viscera (except the liver) removed 25% of the infused Intralipid®. The figures for the per cent removal in the myocardium and subcutaneous tissues were about the same 13% in both tissues. The sum obtained from the different studies (99%) is as close to 100% as can be expected with regard to the methodological errors involved in determination of small arterio-venous concentration differences as well as all assumptions.

In hypertriglyceridaemia a low k_2 is a common finding as can be seen from data in Table I and from several clinical studies. The k_2 value is determined by means of an intravenous dose of Intralipid® and the elimination of Intralipid® is followed for approximately the same period of time during which the Intralipid® removal was determined in studies IV and V. It is therefore of great interest to analyse the data from Table III. According to these data the skeletal muscle seems to play a very important role in the removal of Intralipid® from the circulation. It is possible that an impaired removal process in skeletal muscle may at least partly account for the low k_2 values observed in hypertriglyceridaemia. Althought the present studies do not provide information about the fate of the Intralipid® fat particles removed in various tissues it is tempting to speculate about the possible importance of skeletal muscle in the regulation of the plasma TG pool size and as a possible "critical" tissue for the development of hypertriglyceridaemia.

Clinical studies on the IVFTT (VI, VII)

The relations between the IVFTT k_2 value and serum TG and lipoproteins were analysed in two clinical studies. In study VI healthy female subjects were treated with an estrogen containing oral contraceptive for two months. During this period of time the mean plasma TG concentration rose from 0.92±0.09 mmol/l (SEM) to 1.70±0.11 mmol/l ($p<0.001$). This increase was due to statistically significant increases of TG in all three lipoprotein classes. Although the medication was withdrawn after two months, the TG concentration remained significantly elevated for four weeks. The IVFTT k_2 value remained unchanged by the treatment. The hypertriglyceridaemia thus seemed to be due to an increase turnover of plasma TG probably by a raised hepatic secretion. The role of the IVFTT as an additional tool in elucidating changes of plasma TG turnover will be discussed below.

In study VII hypothyroid patients were studied. It was demonstrated that in the hypothyroid state hypertriglyceridaemia can be found concomitant with the classical laboratory feature of hypercholesterolaemia. Hypertriglyceridaemia generally is due to an increase of VLDL–TG but study VII seems to be the first demonstration of hypertriglyceridaemia due mainly to an LDL–TG concentration increase. It was shown that VLDL–TG was not affected by substitution whereas the LDL–TG concentration values were considerably reduced. When the hypothyroid subjects were compared to a randomly selected material of healthy female subjects of corresponding age it was further demonstrated that hypothyroid patients had a serum lipoprotein pattern which resembled a type III lipoprotein pattern since the cholesterol/TG ration in VLDL was increased whereas

Table III. Intralipid® removal tissues. Estimated plasma flow fractional removal rate and per cent removal of infused Intralipid® in these tissues. The data were obtained from four different studies and each study was calculated seperately

	Estimated plasma flow ml/min	Fractional removal rate % of arterial concentration	Removal of infused Intralipid® %
Myocardium	180	6	14
Splanchnic viscera	(Determined individually in study IV)	5	25
Skeletal muscle	720	10	47
Subcutaneous tissue	300	6	13
			99

"floating β" could not be demonstrated. After substitution the cholesterol/TG ratio in VLDL was normalized. Hypothyroid subjects had significantly lower k_2 values before than after substitution. When k_2 was plotted against VLDL–TG in patients with hypothyroidism a comparison with hypertriglyceridaemic control subjects revealed that hypothyroid patients seemed to have a reduced plasma TG turnover in spite of their serum TG concentration increase.

k_2 and plasma TG fractional turnover rates

The significant hyperbolic correlation between k_2 and plasma TG concentration could be explained either if the high plasma TG had been induced by defect removal mechanisms or if the injected Intralipid® competed with endogenous plasma TG about the removal processes. According to the former explanation the low k_2 obtained in hypertriglyceridaemia would reflect an existing impairment of TG removal processes while the latter concept indicates that the low k_2 in hypertriglyceridaemia was caused by the hypertriglyceridaemia in itself without reflecting the existing fractional turnover rate. According to this concept an increase of the plasma TG pool size will result in a decrease of k_2 and vice versa. There is however evidence that the fractional removal rate k_2 is a process that may determine the TG pool size. Hallberg has demonstrated that the fractional removal rate is not affected by the constant infusion of Intralipid® with various infusion rates (40). In a study by Chait & al (41) patients with alcoholic lipaemia were studied with the IVFTT on admission to the hospital and reinvestigated when their lipid levels were normalized after treatment with diet. Although TG was markedly reduced after alcohol withdrawal k_2 did not rise. In study VI k_2 was carried out in healthy female subjects before and during treatment with an oral contraceptive containing an estrogen derivate. The TG concentration rose significantly but k_2 remained unchanged. In a study by Gustafson and Sannerstedt (23) k_2 increased significantly after treatment with a lipid lowering drug although the plasma TG concentration was not significantly changed. In a recent study by Nestel the plasma TG concentration was manipulated by various diets (42) and the clearance rate of Intralipid® was studied. Although the plasma TG concentration was doubled after five days of an 80% carbohydrate diet the Intralipid® clearance remained unchanged. From these studies it seems reasonable to suggest that k_2 is not merely a reflection of the plasma TG pool size. This suggestion is further strengthened by the finding in study II where in fact a significant positive relationship between the k_2

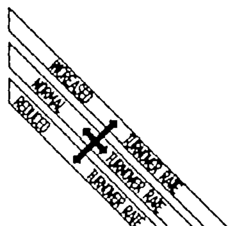


Fig 4 Model for the relationship between k_2 and plasma TG at different turnover rates. The hyperbolic relation between k_2 and TG corresponds to a straight line in a double logarithmic system. When k_2 and TG vary in such a system they vary inversely and thus the turnover rate is constant as long as the product line of these variables is found along the same turnover rate line

value and the fractional turnover rate of endogenous TG was demonstrated

The relationship between k_2 and the plasma TG pool size can be analysed by means of Fig. 4 Such an analysis of the relationships between k_2 and total plasma TG or VLDL–TG cannot yield information about the amounts of TG turned over per time unit. However such an analysis may assist in understanding the mechanisms that may operate in the regulation of the plasma TG pool size Under the assumption that k_2 reflects endogenous plasma TG fractional removal rate (Study II) changes of k_2 and plasma TG concentration can in principle lead to three different turnover results.

1) If k_2 is increased concomitant with a fall of the plasma TG concentration a shift will be observed along the "normal turnover" line The multiplication of total plasma TG by k_2 is a very crude estimate of plasma TG turnover and certainly does not take into account heterogeneity of the various TG containing lipoprotein fractions. Furthermore the k_2 values were higher than the corresponding values for fractional turnover rate of VLDL-TG in study II However bearing these limitations in mind the change would indicate that the improved peripheral elimination of TG results in a reduction of the plasma TG concentration The turnover of plasma TG is however not changed These changes of k_2 and plasma TG have been observed in previous studies on the effects of lipid lowering drugs such as nicotinic acid oxandrolone and clofibrate derivatives (21 25 43). In Fig 5 this pattern is demonstrated with data from ref 21

2) If the k_2 remains constant but the TG concentration is increased, other mechanisms than a reduction of the peripheral elimination rate at least partly lead to the observed increase of the plasma TG pool size Such a mechanism is described in the study of the effects of an oral contraceptive containing estrogen (Study VI) and is summarised in

Fig. 6 This mechanism has also been demonstrated in the study by Chait & al on patients with alcoholic lipaemia (41). *If the* product of k_2 and the plasma TG pool size is used as a measure of the turnover an increase is found. It is possible that stimulation of *hepatic secretion of plasma TG into* the circulation causes this effect (44).

3) If k_2 is reduced and the plasma TG pool size is normal or slightly elevated, the $k_2 \times$ TG product will be low. In study VII *VLDL–TG and k_2 in hypothyroid patients* were slightly lower than observed for a group of hyperlipoproteinaemic females. Substitution with thyroid hormone changed *these data towards normalization. In hypo*thyroid subjects it is thus possible that the turnover of VLDL–TG is low.

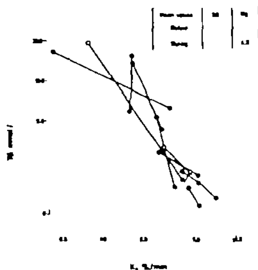


Fig. 5. Relationship between k_2 and plasma TG in ten male patients with hyperlipoproteinaemia before and during treatment with 4.5 g nicotinic acid/day. Mean values of k_2 and TG before and during treatment are given. Logarithmic scales.

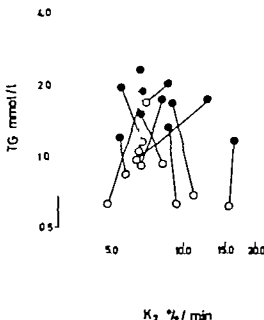


Fig. 6. Relationship between k_2 and plasma TG in twelve healthy female subjects before and during treatment with an oral contraceptive agent containing *an estrogen derivative. Mean* values of k_2 and TG before and during treatment are given. Logarithmic scales.

GENERAL SUMMARY

1 A routine method for the intravenous fat tolerance test (IVFTT) was developed using the fat emulsion Intralipid®

2 When 1 ml 10% Intralipid®/kg body weight was given, first order kinetics for the removal of the fat emulsion were generally found and linear curves were obtained in a semi-logarithmic system.

3 The IVFTT k_2 value (fractional turnover rate of Intralipid®) was highly reproducible when repeated in the same subjects with intervals of up to six months

4 There was a statistically significant positive *relationship between the k_2 value and the* fractional turnover rate of endogenous triglycerides (TG) Furthermore there was a significant negative relationship between k_2 and very low density lipoprotein TG

5 In subjects with various types of hyperlipoproteinaemia and with wide ranges of k_2 values and serum TG concentrations, statistically significant negative relationships between k and TG were observed The statistical significance of these relationships was considerably improved when the relations were studied in double logarithmic systems.

6 The arterio-venous concentration differences of Intralipid® during a *constant infusion* were studied in several tissues and the following results were obtained Intralipid® was significantly removed in the myocardium, the splanchnic region the forearm skeletal muscle and subcutaneous tissue No net removal was observed in the liver If the figures obtained from these studies were extrapolated to whole body removal of Intralipid® it was possible to account for 99% of the total Intralipid® removal

7 The Intralipid® removal in skeletal muscle was studied for the same period of time during which the Intralipid® removal was followed by the IVFTT During this period of time almost 50% of the Intralipid® was removed in the skeletal muscle This major role of skeletal muscle suggests that the negative relationship between k_2 and serum TG concentrations may be due to a decreased TG clearance by skeletal muscle in hypertriglyceridaemia

8 In hypothyroid females hypertriglyceridaemia due to an increase mainly in low density lipoprotein TG was demonstrated

9 From the findings in the present series of studies it was suggested that the IVFTT k_2 value is not a reflection of the plasma TG pool size but rather a process at least partly involved in the determination of the plasma TG *concentration*

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Supplementum 566

Intravenous Glucose Tolerance and Early Insulin Response

Studies on a random sample of women aged 50 and in patients with diabetes mellitus

By Goran Blohme

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FROM THE DEPARTMENT OF MEDICINE II
AND
THE DEPARTMENTS OF CLINICAL CHEMISTRY AND
CLINICAL NUTRITION
UNIVERSITY OF GÖTEBORG GÖTEBORG SWEDEN

Intravenous Glucose Tolerance and Early Insulin Response

Studies on a random sample of women aged 50 and patients with diabetes mellitus

By Göran Blohme

in collaboration with
Ragnhild Arvidsson Lenner
Johan Waldenström

Göteborg 1974

To B bro
Chri tina
and Magnus

CONTENTS

Th l a g n ral con n u of pini n that th p val n of lini ally manif st di bet s m llitu l inc a ing in mo t pa ts of th wo ld (51) A numb of fa to h v b en blamed fo th inc a s including imp oved tandard f living and alt red g composition f th popul ti n.

Sc ening ampaign f di b t s and mo f qu ntly occurring egular h alth ontrols hav undoubtedly ev aled an inc a ed numb of p viously unknown ca of mild d ab t p c ally among th ld ly About 1/3 of th al numb of diab ti a onsid ed to b unknown at g v n point f tim (52)

Th p al n ot manif t diabete s strongly g d pend nt spe ially in wom n (52 72) It h been alcul t d that bout 10 p ent f women and 5 pe ent of men in Swed n will d v lop manif t di bet s du ing th lif tim (72) Still m subj t would hav be om d b tic f th y h d not died from th cau

Th high total mo bidity fo diabetes and th w ll known l t mpl tion of th di a as w ll as th a ociati n betw n diabe t and th oscl rotic vascul d a (63 129 154 166) ind at th t d abete on t tut a m jo h alth p oblem n w t rn countri M thod n ed d f ts ly d t tion and p venti n

Th manif t f rm f th d a s ns d red to p ent only a v ry mall p t f th di b tic ynd om which als includ mino deg f impairm nt f th glu os homeo ta a well th o alled p di b ti tat (51) La g eening inv tig ti n hav ndi ated that an imp d glu o tol an i not un omm n in th gen al popul tion pe ially among th ld ly (25 52 83) Whil g ly impai ed gluc t l nc gen ally a pt d to indi t a d beti tat th gnif an of bord lin tol an fo th f tu e d v lopment f manif st diabete qu lu dati n by l ng te m follow p tudi (29 131 133)

In ul n ti n tudi ha be n wid ly u ed in th inv tigati n of th ly tag of the di beti synd om du ing r cent y a (105 138) I t t has be n focus d n th a ly pha of thi s tion in tudi on ubjects w th minor d g of d turban e in th gluco

homeo tasl or a gen tic predi position for d ab tes owing to th hypothesis that all stages of th diabetic ynd ome including the pr diab tic tate might be cha act rized by a low and d layed ea ly r pons (35) Few data are vailable ncerning the distr but on of th magnitud of the early in ulin espon and th p eval nc of sub normal sponses in the gen al population Cond t on fo populati n tudies ar good in Sw d n a repr s ntativ and up to date sampl of th population an ea ily b obtained from th Revenu Off c R g t r Experi n f m p ev ous populat on studies n Göteborg Sweden indicat that a h gh pa ti pat on at may b anti p ted (77 163)

The pr ent c o tional tudy of wom n g d 50 part of a la g r population tudy of m ddl aged wom n randomly s l t d from th g n al populat on n Gotebo g Sweden. A la ge numbe of es h projects have b n p rform d o ar und way a outl n d by B ngts son t al (14) R sults hav in pa t be n p s nt d ls wh g by B ngts n (13) and Hall trom (78)

Th p nt inv st gation of andomly l t d wom n w suppl m nted by a tudy of m ddl ag d pat nt w th matu ty on t d b t l ted t om th am a a th populati n ampl

Th ma n purp of th p nt in t g tion in w m n ag d 50 w

1 to tudy th gl cos tol an by m ans t an int av nou gluco lo d and to tum t th p val n of impa d tol an

2 to tudy th a ly glu os tim lat d in ulin po a fl ct d by n as in n ulin on nt at in th p ph al blood and to timate th p evalen of a l w pon a n n patient with man t t d abet and in w m n with impa d gl o tol anc

3 t tudy th t on b tw n th n ha d th fam ly h sto y t d b t s and n th oth th gl os tol an and a ly in ulin pon

4 to tudy wh th d ta y h b t and body ompos t on m ght influ n glu tol an and ly in ul in pon

5 to obtain a ba f p o pect tudy w th th p in pal aim of a ng th g an l bn mal a ly n ul n pon fo th f tu d lopm nt of man f t d ab t

Ch pt I

MATERIAL AND METHODS

Mat i l

Th p sent inv tig tion wa p f m d in Göteborg Göt borg th co d la g t ity in Sw den It i itu ted on th w t co t and h d about 445 000 inhab tants in 1968 Furth d tails n ning Göt borg hav b n pr ented l ewhe (60 163)

1 Th p pulation ampl

A and m ampl of wom n in th g t ta 38 46 50 54 and 60 w btain d f m th Rev nu Off c R g st (14) Th ampl onsist d f wom n bo n n dat which w v n mult pl of ix (6 12 18 24 30) Wom n g d 50 (born 1918) in total 436 we l t d fo th p sent inv tig t on. A pa t c p tion r t f 91 p ent w n t d t the p im y examin tion (14) A mall sub ampl (n 20) f w m n ag d 50 h d taken p t in pil t tudy bef r th ma n pop l tio tudy sta ted in a tumn 1968 Thi b ampl w n t in luded in th pr s nt inv tigati n c n rning intrav nou gluco s tol anc and ly insulin r sp n Th total ampl fo this p rt f th tudy n equ ntly con i ted f 416 wom n (T bl I) A total of 352 wom n without manif t di b t w tudi d which r pond to a pa ti ipation at of 85 p r c nt and 87 p r nt of w men avail bl f study

T bl I Th popul t on ampl of women ag d 50

	n	%	n	%
P ti pants	352	84 6		
N n p rtic pant	64	15 4		
D bet m llitu			5	1 2
U em a			1	0 2
D d			3	0 7
Mo ed f om town			4	1 0
R fu ed to p ti ip t			51	12 3

V om n ag d 50 bo n on th 6 th day of v n months w r sel ct d fo the inv stigation of di tary habits tog th with wom n with impai d glu os tol ranc and/o submormal insulin spons a d fin d in chapt VII This will be p esent d in more detail in that hapt r

Th wom n in th p pulation ample had been xt ns vely studied at th primary examinati n (14) All wom n taking p rt in th p s nt inv stigat on had normal rum cr atinin l vel Th qu stionn i s nd th phy cal xamination did not v al cur nt liv diseas in any of th women. No liv fun tion t sts w e p rfo m d in thi ag trata how v r Th p evalence of angina p to i wa 3 per c nt and orona y ECG' 1 pe c nt (13) El v n pe c nt of th women pa ticip ting in th intrav nou glucose tol rance t st (IVGTT) had a blood p su ex eding 160/95 in th s at d p ti n at th primary xaminat on (13) m sur d as c mm nded by WHO (7) About 5 p r c nt of th wom n w e t ated with alu eti diu tic owing to hyp r t n n during th p r od befo e the IVGTT

S rum l p ds w r analy d in all women D tailed port on rum chol t l and rum t gly r d on ntr tion hav b n p s nted l wh (13 15) Th um chol t ol on nt ation anged f m 154 5 to 492 5 mg/100 ml with a mean of 276 mg/100 ml and a l v l f th 9th d c ntile of 335 mg/100 ml Th rum t gly id con c nt tion anged from 0 42 to 4 94 mmol /l w th a m an of 1 26 mmol /l and a l v l f r the 9th de ntil of 1 85 mm l /l

Non of th women wa taking o al nt c ptiv s

Som furth data on th mate al is pr ented in tabl II

n p t p a n t s A few of th ampl of 416 wom n h d died o h d m d aw y f om th town du ing th int rval b tw en th ampling d th nt nded xam nation d t (Tabl I) A mall g up of w m n (31) f ed to parti pat in th f t exam nation F v wom n w th man f t d ab t v f d at th f st xamination and on w man w th em a w n t studi d fu th An th 20 wom n am n d t th p imary a on d d n t g to pa tic pat in th ub eq t IVGTT Th m an lativ body w ght of th wom n who fu ed t p t p t in th p ima y xamination and/or th IVGTT (n 51) w 105 0 ± 20 4 p nt (m an ± S D) s omp ed with 108 7 ± 16 5 p c t (m an ± S D) fo th wom n wh p t p ted

T bl II D t on p ti pant and f s t th IVGTT MD m sing data

		n						X^2	p
			Unm i d	M ed	Divo ed	Wid w			
M tal tat (%)	P ti ipant	352	8 0	77 8	9 4	4 8			
	R f	51	19 6	54 9	13 7	11 9		14 1	< 0 005
			Number f hildren						
			0	1	2	3	≥ 4		
P ity (%)	P tic pant	352	18 8	21 3	35 2	16 5	8 2		
	Ref r	51	29 4	23 5	29 4	7 8	9 8	5 3	> 0 1
			B by ≥ 4 5 kg						
La g babi (% f wom n with child n)	P ti pant	286	10 5						
	R f r	34	5 9					0 7	> 0 1
			M th f th r bling			Oth l ti			
Family hi to y of di b t (%)	Pa t pant	352	19 6			12 8			
	R f s **	48	10 4			8 3		3 7	> 0 1

* MD 2

MD 3

n the IVGTT (t 1 4 p > 0 1) Single women were ov r pre ented among th fu s (Table II) I o ignifi ant diff en s w e noted howev in numb r of child n f equency of la g babi (≥ 4 5 kg) or f q ncy of family h to y of d ab tes b tw en participating women and fu r Th p rti pation ate n th tudy of di tary habits will b di cu ed in chapt VII

D ath tif at w r obta ned from th Cent al Bur au of Stati tic Stockh lm f all wom n bo n in 1918 who had di d in 1950 o l t and wh b au of th ampling m thod w uld hav been inv ted to th examin tion if till al F ft en wom n had d d du ing th p od 1950 1967 The d agnos s of th wom n a pr nted in Tabl III Th d gno was ba ed on autop y f nding in 73 p cent of th wom n

Tabl III. Cau f d ath f 15 w m n who di d b tw n 1950 and 1967 and who be us of th ampling m thod would hav b n in ted to take p t in th popul ti n tudy

C o d t	n		n
H t d		Can	4
T	1	D of n ous y t m	2
al th p t	1	S d	1
F a		A d nt	1
D t n ll t	1		

B th m thod of l t on th popul tion ampl s d t p tativ of th g al population of women 50 T h gh p t p ti n at and ompl ten of information t m t p tin wom ensu that th ults obtained a p t sam popu lat on

II Th mat ial f diab t c pati nt

Pati nts in th of an outpatient linic fo d b t w t d into thi study if th y fullfill d th f ll wing c it ia.

1 Th y w ag d 40 62 (born 1910 1932)
2 Th y w f d to th lini be au f glu su ia with without clinical ymptom of di b t
3 Th y h d on o ep tedly a f sting p ll y blood glucos on nt tion x eding 120 mg/100 ml
4 Th y h d n v b n t ted with in ulin
5 Th y had not b n tr t d w th oth medi am ntal th py f diab t du ing th l t th month
6 Th y w th rw in good h alth and with a tabl body w ight

A o ding t th it ri 34 p ti nt w th mild o mod at m tu ity on et d abet w found all of whom p ti pati g in th p nt inv t g tion Seven p tients h d hype ten i n and w tr ated with a β bl king ag nt (n 5) m thyld p (n 1) o fur emid (n 1) On patient had p viously uff d myoca d al infar tion and n had suff d f m an th l t t o i l hu ight femo al a t ry

Comm nt Middl ag d p tient with diab t w e l ted f th p nt study a th l m un tai ty g ding th influe of ag on insulin tion a eviewed by Johan en (96) Th l cted pati nt m y b ga d d a partly p nting v ry mild f rm f matu ty-on t d b t Th m t al will be di us d m in d tail in chapt VIII

M thod

D f n t on

Manif t di bet m ll tu in wom n le t d fo th p nt popu l tion tudy w n d ed p nt f th woman bef th tudy h d be n p bed d t and/o medicam ntal th py by phy an and at th tudy had a f ting nou bl d gl o o t ti ed ng 120 mg/100 ml On w man w th p ou ly unknown di b t with a f ting blood gluc c nt ti n x eding 200 mg/100 ml n pea ted analy w al n d ed to ha manif t di b t A l ti

was con d red to hav or hav had manif st diabet s if p escribed d t and/o medi amental th apy by a physician.

A clo famil l histo y of di bet s was on id red pr s nt in wom n with a diab tic moth fath r or ibling Wom n with a diab ti g andpa ent aunt un le fi st ou in half b oth (n 2) or own child (n 2) w re g oup d tog th as other relativ in th analys in hapt II and VI In wom n who did not know about their r lativ the f mily histo y was cla sified as n gativ

P ma y populat on study

Th examination outin at th p imary population tudy h been pr nt d p viou ly (14) Info mation con rning m ital tatus wa obtain d from th R v nu Offic R gi t Information on numbe of h ldr n l ge babi s and family hi to y of di b t w obta ned f om qu stionnai and ch cked by int vi w in wom n parti ip ting in th IVGTT Inform tion conc rning th pa nts ag s and if d ad u of d ath w obtained f m qu tionnai Th g of living p rent w noted at th tim of t dy If a p nt was d ad th ag at d ath w noted

Th nt av nou glu o tol an e t t

Th women who w r subj t d to th IVGTT w all ambulatory nd am to th labo tory in th morn g aft f t ng 12 hou A d t w th a a bohyd ate o t nt of about 300 g w pr ec b d du g 3 day b fo th t t. Th wom n r c i ed a written food ch d l p ify g th amount of bohydrat to be on umed at th var ous m al Th impo tan f th a bohyd at f oding w s und li ed Th a t m j ty of the w men stat d at th IVGTT th t th y had follow d th di t chedul Drug w r withdrawn for at l st on d y and smoking wa not allowed du ing th last 12 hours b f th t st A p l q t om wa a s gned fo th t sts and th wom n w r kept th umb nt po t on th oughout th t t. An indwelli g needl w pl d an ant ub tal v in in ch arm and kept pat nt by l w n on ol l Aft an q il br um tim of 15 minut glu 0 5 g p kg body w ght luti n ontain g 50 g glu o per 100 ml w j ted int nou ly du g 5 3 0 minut Immed t ly b f and t 4 6 and 8 m ut aft th tart of th glu os nj t n n blood ampl w tak n n th oppo t arm f r um

in ulin and whole blood gluc e d t rmination Betw en 25 and 60 m nut v nou blood ampl w tak n ev ry 5 minut fo blood glu o d termination

Blood glucose on entrati n wa d termined in dupli at in whol blood by m an of a gluc s oxida m thod u ing a comm cially avail bl g nt (Glox® AB Kabi Stockholm) and with glycin buff d pe chloric a id a p t in p ecipitating agent (111) Th m thodolog cal r ($\sqrt{\frac{d^2}{2N}}$ d diff n betw n dupli at N numb f amples) wa 1 6 mg/100 ml Th co ff cient of va iati n ($\frac{S\ D\ \ x\ 100}{m\ an}$) wa 2 0 p nt at th 225 mg/100 ml lev l alculated f om a f en tanda d rum The bl od ampling and th d terminati n of blood glu o c ncent ation w in all in tanc a i d out by a p ially signed a i tant

Fasting venou blood glu o oncentr tion wa d t rmined in w men at th p imary xamination of th population tudy by m ans f a fe ri yanid edu ti n m thod (88) adapt d fo autoanaly by T chni on N 26 Th m thodological r wa 3 0 mg/100 ml C pill ry blood glu on ntration wa d t rmined in th di betl patients (chapt VIII) at th outp tient clini in th p riod befo and afte th IVGTT by m an of an o Toluidin m thod (81) Th m thodological e ro wa 4 5/100 ml

Th in in blood gluc ntration f m fa ting l l at 4 and 6 minut s aft th ta t of th glu os injection wa us d to alcul t

) th m an of n ements at 4 and 6 minute (G_{in})
b) th m an of in em nts p minut during th f t f v minut (G_{tum})

Wh n alcul ting th G_{tum} t was a sumed that th blood gluc on entr ti n in ed in lin f shi n f om 0 to 4 minute (162) Th 5 minut n nt ation wa obtained a th mean of 4 and 6 minute n ent ti n

Th gluc tol an wa exp sed a a k valu ep s nting th di ppea an f blood glucos in p cent p minut Th k valu w obtained f om th lop f total blood glu on a logarithmi cal f m venou blood glu o con nt ati ns b tw n 25 and 60 minut a ginally d c ibed by Hamilton and St in (79) Th be t

fit of th t aight lin was d t rmined u ing th m thod of l ast squa es Blood glu ose concent ation 10 mg/100 ml o l s ab v th fa ting l v l w r ex lud d becau th blood glucose had th n reach d its st ady tat An exc ption to this rul f r cal ulation of th k valu was mad in four women w th extrem ly high gluco disappea an e at s Th l k valu had to be calculat d f om data points even below f st ng l v l + 10 mg/100 ml (k valu s 4 96 6 70 6 72 and 9 86 p ctiv ly) Th valu tion of th k valu tog th with sults will b discu sed in d tail in ch pt r III

S rum in ul n con entration wa d t rmined by a doubl antibody m thod originally d c bed by Hal and Randl (74) u ing a ommercial adi immuno as y kit (Th Radio hemi al Cent Am ham U K) Th tand d curv wa on tru ted u ing human nsulin d liv r d w th th kit In ulin w d t rmined in dupli at in th p pul tion ampl and in t pli at in the diab ti pati nt Th m thodolog cal ro at th 20 mU/l l l cal ulat d from dupli t d t mination wa 1 4 mU/l in th populati n tudy and 0 9 mU/l in th tudy of d ab ti pati nt At th 50 mU/l l v l th co sponding v lu w r 3 a d 2 mU/l sp ctiv ly Th co ff i nt of v riation wa f und to b 11 p nt at th 17 mU/l l l

Th a ly in ul n pon (ER) was cal ulated a ording to Th r ll t l (162) us g th formula

$$dI/dt \quad f(t) \quad kI$$

wh

I	in ulin con ntration (mU/l)
f(t)	r l a at (mU/l/m n)
k	limin ti n at on tant (f a t on of pla ma pool/min)

Th t mul h be n dev loped as um g th limination of in ulin f m th pe ph al ulat on to b a f t o d p o In th pr s t tudy t w t to 8 m nut A k valu of 0 1 wa u ed corr po d g to half l i (T½) of in ul of ab ut 7 minut s Fo p a ti al pu po th fo mul b d loped to (162)

$$ER \quad {}_8 \quad 0 \; 1 \times a \quad {}_{0\;8}$$

wh 8 th in ul n o nt ation f om th ba al l v l t 8 m t and 0 8 th a b l w th lin u bov th b al l l Th 0 8 wa al ul ted by pp imating th in ulin r w th t ight l b tw th p int 0 4 6 d 8 minut

D ta fr m nsulin analysi w r mi ing in on of the 4 6 or 8 minut amples in v n wom n. Th on ent tion w calcula ted by intra o xt apol tion.

Th sti mation of th m gnitud of th arly insulin spon will be fu th r di cu d in chapt IV The a on f thi i that ult p ented in that chapt r will b of impo tanc fo th choic f m thod u d to int rp t th pon

Th d g f be ty w exp ed as l tive body w ght cal ulat d a p nt f d al w ght' obtain d f om a h ight w ight tabl (112) Body w ight and h ight of wom n not pa ti ip ting in th IVGTT w m u ed at the p im ry xamination (n 20) o w obtained f m qu ti nnai o telephon int rvi w (n 31)

Th m thod u ed f di tary hi tory and body ompo ition in th tudy of di ta y h bit will be p nt d in ch pte VII.

Comm nt

Th h f th intr venou m thod to t t the glu o tol anc wa d t rmin d by th n ed f an int av n u gluco load fo th study f th a ly insulin spon Although th o al way of administ ti n of glucos mo phy logical th glucose di pp ance at ft an ntr nou glu os lo d ha b en on id ed to b a c nvenient measu of a subj t ff n y in di po ing of a glucos load Compa ativ tudi h ve, h wev hown a high in den of di gr m nt wh n th di gno f diabet mad on th b i f an o al gl t l an t t a ompa ed to an int venous glu o tol an t t, d u ed by Ol f ky t al (127) It bviou th t th two m thod will g v diff r nt inf rmation bout a subj t ability to a t during a p od f hypergly em Wh ch m thod m t llabl fo predi tion of fu th d lopm nt f manife t diab t n de elucid ti n by means f long t rm p sp ti tudi s Th m thodolog al backg ound fo th m thod u d in th p ent tudy ha been ar fully di cu ed by Lun ll (117) Peh son (137) and Wahlbe g (166)

Th th o et cal and exp imental backg ound fo th m th d u ed to al ul t th m gnit d f the ly insulin spon (ER) has been p nt d by Th ell t al (162) and will be furthe di cu ed in chapt IV

Statlstl al m thods

Conv ntional m thods w e us d to estimat mean value standa d d viation (S D) t dard er o (S E) and correlation co fficient ( ) (20) By m ans of th s transformation acc ding to Fi h it w t sted wh th r th o r latlon co ffici nt signif c ntly d ff d f om z o (20) St pwi multipl regr ssion was applied to inv t g t the lin r d p nd nc betwe n k valu and ER sp ctiv ly and G_{dos} G_{incr} G_{stim} and l tiv body w ight (53) On way analysi of vari anc in som alculati n comb ned with Sch ff s m thod fo multiple compa i n wa u ed to t st th hypoth i con ning the exp cted valu s in sev al populations (F) (0) Th hypoth sis conc rning xp ted valu s in two popul ti n was te ted by m ans of th Stud nt s t t st (t) (20) The hypoth is conc rning d ff nc in f qu ncies betwe n populations wa t t d by m an of th hi square t t (X^2) o by m ans of th binomial d t ibution with a normal pp oximation ( ) (20)

Th diff nc w con id d tati ti ally signif cant f p < 0 05 wh p d not s th probabil ty of obtaining th tual sampl out om or a mo xt m ult und r th a umpti n that th tated n ll hyp th si is tru The data w r analy d n p t by m ans of om put r (IBM 360/65)

)

Roman m al f to th h pt of th s m nogr ph Arab c um als f to th t n l t pp 115 1 1

PREVALENCE OF MANIFEST DIABETES IN A POPULATION SAMPLE OF WOMEN AGED 50 AND IN THEIR FAMILIES

Gö an Blohmé

Th gene ally c pted view i that clin cally manif t diab t n titut s only a small part of th di beti ynd om which also includ mino d gr s of impai ed gl cos tol anc a w ll th alled p ediabeti tat (51) Studying women ged 50 a th low f equency of manif t d ab te may be xpe t d as th pr val nc in th general populati n in Sw d n at th s ag h s been tim t d to be about 2 pe ent (72) Th p ent population tudy w h w v mainly d gned to tudy th tag f th diabeti ynd om befo e th manif t f rm of th di a ha pp a ed. A th p evalen of manif t diab t s trongly g -d pend nt (52 72) an impr si n f th total d abeti p edisp tion might b gained by studying th pa ent of th lected women Th main purpo of th part of th p ent inv tigation w to tudy th p val n of manif t di bet in th wom n andomly lected fo th p ent population tudy and in th p nt

MATERIAL

Inform t on co rn ng family h tory f diabet wa obtained f 98 p nt (n 426) of th total ample of wom n ged 50 Th wom n had di d and fou moved way f om th town betw en th amp ling and th examination d t Inf rm ti n on rning family hi t ry wa thu obtain d fo 99 p ent of th wom n avail bl f tudy (n 429) In wom n who did n t know bout th lativ th family h to y w cla fied g ti One p i of twin was pr nt in th sampl Th p rents w a counted fo only on A few wom n ould ot g v inf rm tion on th ag of th i pa ent at th pr ent tim g at d th. Inform tion in thi pa t of th p nt study w thu btained f 97 pe ent of th women vailabl fo tudy conc rning m th r and fo 95 pe nt onc rning fathe

For further details of th material see chapter I
For m thods and definitions ee chapter I

RESULTS

Manife t diabet s in the population sampl

Four of th wom n called to th primary examination had a p eviou ly known manif st diabet s Two of th m we e being treated with in ulin and two had b n pr cribed di t only One woman with pr viou ly unknown diabet had a fa ting blood glucose conc nt ation abov 200 mg/100 ml on r peated analysis In the group of wom n who r fused to participat in th fir t examination none wa aware of manif t diabe te D ath ce tificat disclosed that on of 15 wom n who di d in 1950 or l t r (afte th ag of 32) had manifest diabete Th frequency of manif st diabetes in wom n who we or would have b n ampl d for th ag trata of 50 wa 1 3 pe c nt.

Manife t diab te in th fam ly

Th pr val nc of known family hi tory of diab t was 30 4 p r c nt in women availabl for tudy (Tabl 1) Th wom n port d diabe t s in mothe about twic as oft n as in fath (X^2 12 2 $p < 0$ 001) Thr of fi e women w th man f st d ab t r p t d a moth with d abet s Th prevalenc of d abet s in pa nt who had ched o pas d th ag of 75 wa 15 5 p cent in moth rs and 7 0 p cent n fath s (X^2 7 0 $p < 0$ 01) In th group of par nt 53 p c nt of th moth and 36 p c nt of th fath rs w e living at th tim of th study

Tabl 1 P alen of fam ly hi to y of diab tes in women ag 50 a a labl f tudy

Type f fam ly h to y	n		%	
Clo fam l al hi to y	80		18 7	
M th		49		11 4
F th		21		4 9
S bling		18		4 2
Oth l t	50		11 7	

L ght wom n po ted fam ly h to y in both p nt o on pa nt and bling

Th pa nt ag

Th m an g of moth t th tim of th tudy o at d ath wa 71 3 ± 12 4 (S D) y ar omp ed to 70 3 ± 14 1 (S D) y ar in fath (t 1 1 p > 0 1). At th tim of the tudy 63 p cent of th moth and 79 p nt of th fath w d d som of them having died at ly g (Tabl II) About half of th pa ent had each d th ag of 75 y ars A signifi antly high f equency of f th r had died befo th g of 60 (2 60 p < 0 01) a w ll a bef the ag of 70 (2 35 p < 0 01) ompared to th moth Th maj rity of th s a ly death w a c ib d to inf tious di a malignant di a a cidents uicid Th f qu n y f d ath a bed to heart di a wa n t ignificantly high in fath s omp ed to moth befo th ag of 60 (1 61 p > 0 05) befo th g f 70 (Tabl III)

T bl II. Data on pa nt of women g d 50 availabl for study (n 428) Th ag of living pa ents w not d at th tim of tudy If a pa nt wa d ad th ag at d ath w n ted. MD mis ing d ta

	Ag (y ar)	<50	50 59	60 69	70 74	75 79	80 84	85 89	90 94
M th n 416*	Aliv (n)			2	48	53	35	10	5
	D d (n)	26	30	65	51	48	27	16	
	D ab tic (n)	1	1	7	10	17	9	3	1
	(%)	3 8	3 3	10 4	10 1	16 8	14 5	11 5	20 0
Fath r n 405**	Aliv (n)				14	35	25	8	3
	D d (n)	40	42	67	43	55	58	11	4
	Di beti (n)	1	3	3	0	3	9	1	1
	(%)	2 5	7 1	4 5	0	3 3	10 8	5 3	14 3

MD 12

** MD 23

Tabl III Numb r of d aths ascrib d to h art di ase b fore th ag of 70 in par nt of wom n aged 50 (those sp cifi ally ascribed to myoca dial infarction o angina p cto is also eparat ly in brack ts)

Age (year)		<50	50 59	60 69
Moth	n	2 (0)	5 (4)	21 (12)
Fath	n	5 (2)	9 (7)	13 (11)

DISCUSSION

Th pr val nc of man fe t diab te in th p ent andom sample of women aged 50 was 1 3 p r nt. Although the mat ial i too mall to p rmit lid conclusion concerning th preval n e of diab t in th g n al populati n at th ag th esult i onsistent with that of a ar f l in tigation in 1965 in Sw den (72) In that study the agg gat mo b dity ri k for diab t s up to the ag of 50 wa timat d to be abo t 2 p ent n both exes In add tion the p evalence of mani f t diab t n m n g d 50 n Got bo g Swed n was found to be 2 p r c nt (60)

Th p al n of man f st diabet n Swed n consi tent w th p al n f gu s po t d f om oth r w te n countri (62 100 1 1) Th p al n of manif st diabetes ha b n port d to be fa h gh n rta n populations of fo exampl Ame i an Ind ans (121) and Ind an n South Af ca (27) Thi indic t that local diff nc in gen ti d o n ronm ntal facto may be of ons d rabl impor tan th olut on of th d abeti ynd om Con lusions drawn f om th d t n th p t tudy may thu only b valid fo populat ons mp bl to thn o gin and soc o onomi l vel as in Sw d n nd im l w t n ount

A gen t p ed po t n fo th dev lopment of d abet m llit h lo g b tak fo g ant d d ab t f quently port a family h to y o th d wed by Ha ald (82) The epo ted p f n of fam ly h to y of d ab te al high in diab t th n n d bet (45 99 134 159 169) A h gh pr valence i newly d t t d d bet b t w th than in subj t w th t fam ly h to y o th d h also be n fou d n la g n ng

inv stigation (52 150) Th m t int ating point in the o p evalenc figu i howeve that ven individual without known diabete about 98 pe nt of th gen al pop lati n in we tern countri s h v a high pr val nc of lativ with diabete This s consi t nt with th total pr valenc of 30 4 p ent in th p sent tudy mainly com p i ing women without manif t diabetes Thi und lin th fact that the av r g pr valenc of di bet in th total population (1 2 p c nt) doe not fl t th h gh total m bidity f th di a du to th fact that th p val nc is strongly ag -depend nt (52 72)

In th p e ent tudy as many as 11 4 per nt of th women po ted a diab ti moth and 4 9 pe c nt a diabeti fath Many of th non di beti pa ent h d died at ag wh n th preval n f diab t s is ath low Fu th rmor about 1/3 of th moth s and 1/5 of th fathe w e till living at th dat f the tudy and m y develop diabe tes lat n.

The aggreg te m bidity isk and life tabl xp ctan fo clini ally manif t diab t s in Swed n in 1965 was calcul ted by Gronberg t al (72) Th agg eg te morbidity k fo wom n in a d in that tudy f om 1 9 pe ent t th g of 50 to 10 5 pe ent t g of 75 and to 13 0 p cent at th ag of 90 Th ponding figur fo m n w 2 1 5 3 and 6 5 pe nt sp tiv ly Thirt en pe ent fo wom n and 6 5 p cent fo men p nt th total mo bidity ri k of an indi vidual devel ping manif t diabet up to th ag of 90 Howev many of th p umptiv diab ti will not devel p th di a th y will d befo the on t of th di a An exp ion of th p obability that a newborn child will dev lop manif t diabet during hi lif tim i rived at by d t rmining th lif tabl exp ctan fo manif t diab t s Th lif table exp tan y was calculated to b 1 9 9 2 and 10 2 p c nt fo women and 2 0 4 4 and 4 8 per c nt f m n at th ag l vel m nti ned (72) In th p ent tudy m th wh had r ached o p d th g of 75 had a p evalenc of diabet of 15 5 p cent ompa d to 7 0 pe ent in fath in spit of th f t that about 1/2 of th moth and 1/3 of th fath s in thi g oup w till living Th total mo bidity ould be xp cted t in a furth if the mate rial had be n examin d om yea l te Th p di po ition fo di b te in pa ent who had died befo th age f 75 in fo xample diov scul di a e might w ll hav been still high s th fre

quency of impaired glucose tol ranc by d finition chemical or sub clinical di b tes (51) has frequ ntly bo n repo t d to be high in patient with such dis ase (86 135 166) A high frequ ncy of arly death in fathers than in moth r found in th pr nt study wa mainly due to caus s oth than h a t dis as h wev Th higher p valence of diab t s in moth s than in fath r i con ist nt with sult obtain d in oth r ent studi s (52 72 107) indi ating a high diab tic pr di position in women than in men

A in mo t oth tudi s onclusion conc rning the preval nc of a family history of diab t in th pr nt tudy hav b n based on information gain d by int rviews or qu tionnair amo g th probands Som ror oth r than tho di cu s d abov may thu h v b n intro duced in th calculation of th total mo bidity i k fo manif t diab t s A g at variability of th di as p tt rn with a high p opo t n of mild s s sp cially in th ld ly would xplain th high p opo tion of p ously unknown c s (30 40 p r t) found n r n g p ogram m s for diab t s (52 145 169) In th p nt tudy n of s x diab ti pati nt wa p viously nkn wn R porti g of d b t am g r la tiv s dep nd on th p oband knowl dg of th di h to y of th i r lati s In th population tudy n T m h th fam ly h to y d ta for ibling w ch ked n a subgroup (124) Th o ur n of f l pos ti e data wa n glig bl Th s bj t int ew d knew how of only 25 p r cent of th al n mbe of ca of d b t in th i b ling

Th typ of r o di u ed r lt n d stim tion of th al f qu n y of manif st d b t s and m ght cau mark d u d t m tion of th total m rb d ty isk fo th d as Th e thu g und f r a sum g that th t tal morb d ty ri k for d abet s in Swed n will c d th 13 p nt fo wom n and 6 5 p r c t fo m n port d by G ön berg t al (72)

SUMMARY

Th prevalen f man f st di bet in wom n ag d 50 who w r s l ct d fo th p nt population sampl o would h be n l ted f st ll al wa 1 3 p nt On of x wom n h d p eviou ly un known d b t Th p al n of a known fam ly h tory of d ab t w s 30 4 p t. Th p al n of diab t s n moth s (11 4 pe nt)

w s twic that in fath (4 9 p cent) M th who had ach d o p d th age of 75 had a p evalen of diabet of 15 5 p ent. The di b tic p edispositi n in w m n will be ath high a th total mo bi dity k for diab t in women may be a sumed to ex ed th 13 p cent ugg sted by G önb g t al (72)

Roman num al r f to th ch pte of thi monog ph Ar bi num al f to th f en li t, pp 115 121

Chapte III

INTRAVENOUS GLUCOSE TOLERANCE IN A POPULATION SAMPLE OF WOMEN AGED 50

Gö an Blohmé

Population studi in B dford (25) Tecums h (83) Sudbury (134) p t of south rn Califo nia (146) Gr ater Clev land (104) and oth r ar as ha ind cated that hype glycem a d ing oral gluco tol ance t ts is a not uncommon finding n th g n al population sp cially in ld indi iduals Diff en in composition of studied group in m thod of t sting and in the r ter a used for aluation of r sult may be pon ibl f r the diff enc in ported preval nc f gu a d cu ed by O Sullivan (130) Th nation wid p val n e of hyp gly mia in th U S ha b n e timat d u ing th sult of a h alth xamination surv y ondu ted by th National Cent r fo H lth Stat stic on a pr s ntativ ampl of th U S ad lt population (122) In th g g oup 45 54 8 2 per ent had a blood gluco co c ntr tion abo e 180 mg/100 ml on hour aft ng st on of 50 g of glu o In th g g oup 75 79 th p al n wa h gh as 29 5 p r c nt.

Th int av nous m thod fo d t m ning th tol ran to glu o ha m inly b n sed in lim ted group of subj ct this um stant l t hnique s une itabl fo mass s e ning No th l ss th e l g of healthy ndi d al ha e been stud ed in Sw d n d ng c t yea u ng th s m thod (85 117 166) Only on of th m how e was p rform d in subje ts andomly s lected f om th g n al population (85) In th t study only m n w r ncl d d No d t a a a l bl bout th pr alenc of an imp r d t l anc t an int ou gl s lo d n m ddl ag d wom n rand mly sel t d f om th g al popul tion

Th m purpo s f th s p rt of th p t n stig t on w to tudy th f q n y d st bution of th glu o d s pp a an e at d s f t il ng th a bl d stimat th p al n of mp ed gl t l ran

Fo m t l d m thod h pt I

Fasting blood glu os

Fasting v nou blood gluco on entrati n tended to be n rmally di tributed in women without manif t diab te with a m an valu of 70 2 mg/100 ml (Tabl I) A w ak o lati n wa f und b tw n fa ting blood gluc c n ntr ti n and lativ body w ght (0 28 ± 5 37 p < 0 001) A ignificant in r ae in f ting blood glucos lev l with in ing r lativ body w ight wa al o hown using on way analy i of varian (Tabl II)

Tabl I Blood glu n entr ti n befo and afte an int a n u glu o lo d in 352 wom n ag d 50 G_{inc} m an of in m nt in bl od gluc at 4 and 6 minut G_{tim} m n of increm nts per minut du ing th fi t fiv minut

	Blood glu mg/100 ml			
	M an	S D	Rang	
0	70 2	8 3	52	107
4 min	320 2	53 0	139	465
6	323 4	34 6	205	414
8	303 3	30 9	211	451
G_{in}	251 6	37 9	120	363
G_{tim}	150	29 1	44	231

Glu o timul t on

A wid ang in blood glu o n ent ation wa obs ed aft r th int nou gl load. Thi wa vident in th total mate ial (T bl I) w ll at diff nt l l of elativ body weight (Tabl II) Th glu o timul tion exp ed as G_{in} and G_{tim} thus v ried m kedly b tween th women A ig ificant inc ea e in th va i bl wa n ted with inc a ing lativ body weight u ing on way analy is

T bl II F t g bl od gl co and blood gl cose timulation in 352 wom n subg ouped according to l t body w ght Fo ymbols s tabl I

R l t body w ght	n	Fa t g blood glu o mg/100 ml		G_{1} cr mg/100 ml			G tim mg/100 ml		
		M an	S D	Mean	S D	Rang	M an	S D	Rang
90	34	66 8	8 3	1 8	34 8	141 297	131 1	27 2	64 173
91 100	77	68 4	6 9	238 1	34 9	137 319	142 6	.7 1	79 195
101 110	106	69 4	7 5	250 6	37 3	120 348	149 0	30 3	44 231
111 1 0	70	71 9	8 3	61 1	33 9	164 327	157 0	26 0	89 212
1 1 130	31	73 5	8 5	64 1	27	194 316	156 5	4 0	99 02
≥ 131	34	73 8	10 8	83 9	32 9	19 363	170 5	25 6	1 0 30
F_{ob}		5		14 3			9 3		
p		< 0 01		< 0 01			< 0 01		

of va iance (Tabl II) a w ll as reg s ion analy is (0 43 and 0 34 8 59 and 6 62 sp tively p < 0 001)

Glucos d app anc rat

Th di t bution of k valu wa skewed but t nd d to be normali ed aft logarithmic t ansformati n (Fig 1) Th was no videnc of a bimodal di t ibution of th k valu s Th di tribution of k valu s wa th am wh th th mate ial w stri ted to wom n with a l tiv body w ight of ≤ 120 p ent (n 287) or a lativ body w ght of 91 110 pe c nt (n 183)

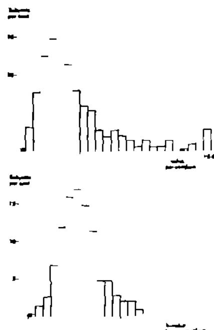


Fig 1 P entag d tribu ti n f th k val es and th l garithms in 352 w m n ged 50

Th k valu va ed ov a w d ang with a m an valu of 1 88 and a med an valu of 1 59 A few women with xt m ly high gl os di pp a an t had alr dy ched th f ting blood gluco l v l w thin 30 35 minut s aft th tart of th glu inj tion. The m an log k al wa 0 2218 rr sponding to k valu of 1 67 The d en til f th distribution f th k valu p s nt d in T bl III Applying t ditional limit fo n rmal and path l gi al k valu s it w found that 6 0 p nt of th wom n had a k val below 0 90 11 1 p ent a k val below 1 00 and 17 3 p cent a k valu below

1 10 Wh n the material was restricted to women with a lative body w ight of ≤ 120 pe c nt th co esponding f qu nci e w r 6 3 10 5 and 15 0 p nt sp ctiv ly In wom n with a relative body weight of 91 110 p c nt 4 9 9 8 and 15 8 p nt had a k valu b low th limit

Tabl III D c ntile of th k valu di tribution in 352 women ag d 50

D centil	k valu	D ntile	k valu
1	0 97	6	1 75
	1 13	7	1 97
3	1 29	8	2 29
4	1 44	9	3 13
5	1 59		

Co r lation

Co lat n co ff c nt near o w r found betw n th k valu and the glu o stim lation xpr d as G_{incr} (0 04 z 0 75 $p > 0.1$) and G_{tim} (r 0 05 z 0 94 $p > 0.1$) pe tiv ly Only 1 4 p r nt f th total v anc n th k val wa xpl ined by th gl o do wh ch n f ct was w akly n gat ly o lat d to th k l (0 12 25 $p < 0.05$) (F g 2)

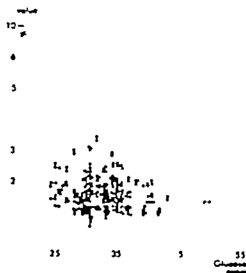


Fig 2 R lationship betw n adm ni t d total dos of gl cos and k val in 352 women aged 50

Th G_{in} and G_{tm} vari d ma kedly ven in w men with adja ent body w ights and r l tiv body w ight In a g oup of women with relativ body w ights of 95 105 p nt (n 110) tho with a body w ight of 60 0 64 9 kg were el cted (n 47) In thi homogeneou group as ega d total do of gluco e and d s of glu os p kg fat fre w ight th G_{in} anged f om 151 t 330 mg/100 ml and th G_{tm} from 77 to 210 mg/100 ml No gnificant correl tion was found in thi group betw n G_{inc} and the k valu (0 24 1.62 $p > 0$ 1) o b tw n G_{tm} and th k valu (0 22 1 48 $p > 0$ 1)

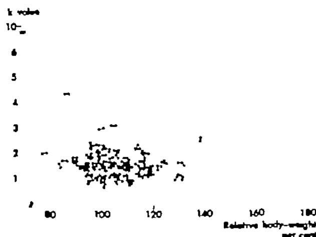


Fig 3 Relation hip b t w n latiy body w ght and k valu in 352 wom n ag d 50

A v ry w ak co r lation wa found b tw n latiy body w ight and th k valu (0 11 2 06 $p < 0$ 05) (F g 3) Thi tenden y f d asing k valu with inc ing latıv body w ght wa furth r shown when th wom n w g ouped a ding to latıv body w ight (T bl IV) Th d a ing k value with in ing r latıv body w ght w howev not s gnif cant u ing on way analy of v i an No could the chi quar method disclos any dependenc of k valu on lativ body w ght when t t d on th whol mat al (Tabl V) Th f quen y of k valu below 1 10 wa howev ig n f antly high in wom n with a latıv body w ight bo 120 p nt than n lean women (2 44 $p < 0$ 01) In addition th fr quen y of k alu abov th la t (ninth) d ntil of th di t ibution of k al s wa gnif cantly low in wom n with a lati e body w ght bov 120 p nt than in l an r women ($x = 2.82$ $p < 0$ 01)

Table IV The k value in th total material and ubgrouped ac o ding to relative body weight

R lati body w ight %	n	k value Mean	S D	Rang
76 170	352	1 88	1 10	0 59 9 86
≤ 90	34	2 01	1 12	0 62 5 62
91 100	77	1 86	0 95	0 59 6 42
101 110	106	2 08	1 37	0 71 9 86
111 120	70	1 71	0 79	0 66 4 77
121 130	31	1 74	1 25	0 85 6 70
≥ 131	34	1 67	0 70	0 77 3 72
F_{ob}		1 5		
p		> 0 05		

Tabl V V om n (%) in th total mat rial and in cla s of lativ body w ight ubg ouped acco d ng to the k valu

R lativ body w ight %	n	k valu < 0 90	0 90 0 99	1 00 1 09	1 10 1 19	1 20 2, 29*	2. 29 3 12*	≥ 3 13
76 170	352	6 0	5 1	6 3	6 3	56 3	9 9	10 2
≤ 90	34	5 9	2. 9	2 9	8 8	52 9	11 8	14 7
91 100	77	5 2	6 5	9 1	3 9	53 2	14 3	7 8
101 110	106	4 7	3 8	3 8	10 4	52 8	9 4	15 1
111 1 0	70	10 0	2. 9	1 4	5 7	64 3	10 0	5 7
1 1 130	31	6 5	9 7	12. 9		61 3		9 7
≥ 131	34	2 9	8 8	14 7	2 9	55 9	8 8	5 9
χ^2		36 1						
P		0 1						

* k lu d til VIII 293

** k al d c nt l IX 3 1 6

A tepw multipl g e n analys wa p fo med to valuat th compound influen of latl body w ight gluco do G_{in} and G_{tim} on th k valu (Tabl VI) Th se f u variabl s togeth ould only explain 2 p nt of th total a nc in k value

Tabl VI Stepw multipl eg i n analy with th k valu th d p nd nt ariabl Fo symbol f a bl table I. Va iabl a l t d in th ame o d th y nt d into th r g n quati n b (o ff nt of g n) and β (o ffi i nt f standard reg on) ar final alu R (f a tion of xpl in d va an) s g n i h t p

Va abl	b	β	R^2
G_{do}	0 011	0 06	0 0140
G_{tim}	0 010	0 28	0 0143
G_{in}	0 001	0 29	0 0193
R l ti body w ght	0 006	0 09	0 0203
Co ff t of multipl g a n			0 14
F t i pl in d			0 02
C n tant t m			2 39

DISCUSSION

Th p n t nal t dy o w men ged 50 howed a wid ang i gl d ppea an at ind ting ma ked diff en c n o all gl o util zati n. Studi n the p odu bil ty f th IVGTT h hown th t th ate f glu o d ay may chang from t m to tim w th th am indi dual (32 56 84 135 137 166) Th gl d e pp an t may b n d d to dep nd int al th amount of culating ul n a w ll n fa to d t n n ng th in ul n n ti ty in p iph l ti u s S v al h rmon w ll di ta y fa tor ha b en d u ed in thi conn ti n (89) A t d d d t t p oc du a w f llowed in th p nt n tig t on m y b n d ed to minim such facto fa a

po sible As n phasized by Faj ns (64) howeve the lack of repro ducibility of an oral glu os toleranc t st in ca s of borderlin gluco tol ra e may in pa t fl ct the n tu al history of diab t s with fluctuati ns th d g of di tu bed glucos homeostasis No r p at d t st w e p form d in the p ent inv tigation due to th fa t that th wom n st di d h d taken part in num ou stud s and fu th r tudi a planned fo the f tu e It may well be that om of th wom n w th a low k val wo ld hav hown an improv ment in gl co e toler c if a p ated t t had b n p form d whil oth r wom n wo ld h v hown a d t io ation.

Th f q n y d st ibut on of th k val e wa skewed with the slop t wa ds h ghe v lu which is on i t nt with res lt obt ined in oth r t di s (11 116 117 166) The continuou and unimodal d st ibution of th k alue in th p nt study as w ll a in p eviou t d nd at s that t s impo bl to e tabli h a l mit betw n d cr ased tol nc to glu o and no mal tole an xc pt arbit a l ly Unim d l d t bution of th blood glu os l v l aft r an oral gl co lo d h s als be n epo t d (19 25 83) The l ck of bim d lity h often b n la n d to p o a mult genic u of th d beti p ed po t Th s hypoth s has be n d uss d and chall nged by St inb g (155) In popul t o w th a y high p ev l of impa d tol ran t gl o dm n t ed o ally a bimodal d st bution of th two hou gl l l h s be eport d (156) Th int rpr t t n of th d ng w that th d bet p ed sp s t on m ght b du to a gl g d th g n al population might b comp sed of two ub popul t on rmal nd one di bet a d abetic popul tion b k o al d d tr but n wh r only a mall p rt consi t d of d b t p t t Th imodal d st ibut o of th k al in th pr s nt t d and th um d high diab t c p edi po it on (II) do n t ppo t th hypoth th t a sp ial populat on with a g n tic lly d t rm d p d po t on fo d b t wo ld be cha t rized by a m k dly low gl o d s pp an at than indi id ls w thout th p d p t o

Th m an k l (1 88) th p t tudy gr d ery w ll w th th m k l 1 91 m n g d 50 domly s l t d f om th p p l t o t Upp l Sw d (85) It high than th m an k alu of 1 58 th w n 34 8 (m an g 55) n th tudy by Wahl b g (166) In th t d f w n g d 3 61 (m n g 46) by L ll

(117) th m an k value wa alculat d f om log k valu The m an l g valu in th t study co sp nding to a k valu of 2 18 w high than th m an log k valu f th p nt tudy sponding to a k valu of 1 67 Th r sult of som oth tudi ha b n eviewed by Wahlberg (166) R ported diffe en in m an k valu might depend n diff n in ompo ition of tudied g up w ll a diff nt t t pro edu Sev al fa to might b c n id d to influenc n th glu di ppea an at om f which will b di cu sed furth

Ca bohyd at f eding Th u of carbohyd t ich di t befo the p formanc of a gluco tol anc t t h s be n subj t to discu ion fo d cad as eviewed by Lun ll (117) and Wahlb g (166) R c nt tudi hav again hown th t ca bohyd at f ding may improv and ca bohydrat tri ti n impai th tol an to an o al glu os l ad in normal subj t a w ll a in subj t w th im pai d glu tol an (6 21 89) Limited data n th ff t of chang in di ta y ca bohyd at n th gluco d ppea an at aft an intravenou gluco lo d sugg st a imil influ n on thi p am t (6 47 89 109 166) Thi ff ct ha been att buted to alt ed n itivity to insulin in pe iph al ti su when th onsump tion of di ta y bohyd t i changed (89)

A p ep ato y di t with a carbohyd at ont nt f bout 300 g wa p ibed during 3 d y b fo th t t in th p nt tudy a a di t t icted in a bohyd t may b a comm n finding in middl ag d wom n esp ially during a w ght educing gimen It annot be xcluded th t som f th women with a bo d lin o low normal k val in th p ent tudy would hav had k valu within th path lo gical ang without ca bohyd ate f eding In th tudy by Lun ll (117) bohyd t ich d t w p ibed bef th t t whil in th tudi by Wahlb g (166) and H d t and (85) n bohyd at p iming w u ed

Ag A dec ing k valu ha b en ep rted with inc ing g in h althy s bject (11 44 117) well as in pati nt with na y h a t dis (135 166) Thi d a ha mainly been att ibuted to an in a ing deg of obe ity with g (116 117) A m ked d a in m an k valu w howev noted when a g oup of w m n in th tudy of Lun ll (117) w t ted 5 7 y lat t a m an ag

of 52 d pit unchanged m an body w ight (118) This indicat s that aging p may be a ociat d with a d rea ing rate of glucos utilization which might d p nd on d rea ing ensitivity to insulin in p riph al ti u with age In the p nt study th ag factor was r umv nt d as only on ag trata w s studied

Ob sity It i g n ally pt d that obe ity i trongly associa ted with th d v lopm nt of m nife t maturity ons t diab te (52 101 164 168) In the p e nt study a t nd ncy to d asing k valu with inc asing lativ body w ight was noted. Thi was mainly att ibuted to a high fr qu ncy of k valu below 1 10 and low f equen y of high k alu in wom n with a l ti body w ght abov 120 p r ent compar d to l an wom n Th as o ati n found b tw en ob ity and a dec as d k valu s in agr m nt w th ults obained in oth st di (11 85 116 117 137) and may b du to a de a i g in lin ensit vity n per ph al t su with n a ng deg e of ob ity as di cu ed by Horton t al (89)

Gluco stimulat on In th p s nt tudy n that of Hed t nd (85) th gluco do w s adju t d o d g to body we ght a gg sted by Ikk and Luft (91) Th gl d p kg fat f w ght th n ased w th in s g d g of obe ty Th glu os tim lat o wa thus high n ob th l an w m In th p s nt study th was flected by an so iat n lthough w ak betw n lat body w ght and th n s in blood glu os n t at o d ng th l t few m nut s aft th glu o nj t on Con d abl d d al ti ns both l n nd ob wom n n blood glu o m nt ft th gl o inj t on w r f nd A w d ang blood gl o m nt was al o fou d n wom n w th body w ght w th a t w kg and lat body w ghts w th n ± 5 p nt of d l w ght Th g w th ult obta n d n p ou ly report d tud s d t g w d g n th olum of d t but of gl o (32) o g f ant o l t was found b tw n glu os timul t d th k al h w th n th t tal m t l o n th g u p hom ou g ds t t l gl os d s and glu os d p kg i t t w ght

A sl ght th k lu w po ted wh th gl o d s wa d i m 5 t 50 g n nt d d al mp is n

(55 166) In oth r studi however an in as of th glu o do had no ignifi ant influen e on the k valu (5 42 117) In th p e nt tudy th glucos d w 25 40 g in 90 p r cent f th wom n The g ssion analy s showed a v ry w ak n gativ o elati n b tween th glu o d and th k val e as well a betw n th lative body w ight and th k valu Th e two va bl ould ach explain only about 1 p cent of th total varian in k valu It cannot b ex lud d however th t the high d of glu o in obe wom n than in l an wom n m ght hav c u ed m wh t high k value in th obes wom n than f a stand d dos of 25 g had b en used Th t nd n y to a dec sing k val with in a ing l tiv body w ight m y thu h ve be n und tim ted Th stepwi mult pl eg on analysi howed how v r th t n th r the d g of ob s ty no th d g f gluc s stimul tion w maj d t minant of th k valu in th pr sent tudy In fa t th va iabl t g th ould only x plain about 2 p cent f th t tal va ian in th k value

P val nc of mp r d gl c tol an

Ba d on tud in pati nts with lin ally manife t di bet a k val e b low 1 00 has f equently b n ega ded a p tholog al a evi wed by Wahlbe g (166) Th lim t w ch n emp r cally Wahlb g (166) found th m an k al pl tw S D in group of p tients with lini ally man f t d ab t to b 0 91 In a view f th l t tu h found that 7 p nt f p ti nts with d bet had a k al of 0 91 high r Th ult f m th diab tic pati nt and th unimodal di tr bution of th k val in th contr l subj ts without ona y h rt d u ed Wahlbe g b tr rily to d fin k valu at o b l w 0 90 p th l g al b tw n 0 91 and 1 10 a bord lin and bov 1 10 a normal (166) In th p sent tudy 6 0 p ent of women had a k alu bel w 0 90 and 11 4 per ent a k valu betw n 0 90 and 1 10 Th f equ n s w e s ntially th am wh th m t al w s t cted to w men with a r lat v body w ght at o below 120 p nt within ± 10 p ent of deal w ght In the study f andomly elected m n ag d 50 in Upp ala Sweden th o ponding f quen w 9 5 and 4 5 p ent sp t ly (65) In th tudy by Wahlbe g (166) 6 p c nt of men and nly on p nt of women had a k valu ≤ 0 90 whil th bord lin f qu w 12 and 7 p ent sp ti ly In Lunell

study (117) none had a k alu < 1 00 When ret ting a ubgroup five yea s lat r 7 p r cent had dev loped a k value < 1 00 howev r (118) It i obvious that factor d scussed abov conc rning differences in compos tion of tudied g oup and in test procedur m ght explain th e d ve g nt r ults

Th re ult of th p s nt st dy indicat that a consid rable number of wom n ag d 50 hav a glucose disapp arance rat which is g n ally accepted to indicat a d ab tic stat Th s gnif canc of a low k valu and of such facto s as obes ty d tary hab ts and family history of diab tes fo th futu dev lopm nt of manife t diabet s needs a pro p cti tudy to b det m ned

SUMMARY

Th p e nt o s s t onal study of wom n ag d 50 how d a wid range n gl o di app rance rat w th a m an k valu of 1 88 and a m d an k alue of 1 59 Th cont nuou and un mod l distribut on of th k alue d d not perm t to establ h a l mit b tw n a d r d and a normal k valu xcept a bit a ly U ing tr d t onal l m t a cons d bl umbe of women h w d a k alu wh h s g n ally acc pt d to nd at a d bet stat S x p nt h d k val < 0 90 11 p ent a k alu < 1 00 and 17 pe t a k alu < 1 10 A w ak a o ation wa found b tw n obe ty d a d a d k alue Th va ian n lat body w ght tog th w th th a n in gl o stimulat n o ld howe only xplain 2 p nt of th total a n n th k 1

Rom n m l t th h t o th m og ph A b m l tt l t pp 115 121

EARLY INSULIN RESPONSE IN A POPULATION SAMPLE OF WOMEN AGED 50

Gö an Blohmé and Johan Wald n trom

A c n id abl amount of w rk ha b n pe f rmed du ing the l t t n y a n th tudy of th in ulin s c ot ry apacity a fl ted by an inc e s in insul n on ntration in th p iph al blood pecially in individual with min d gr of abnormality in th gl co homeo stasi o with a g n tic p ed p sition f diab te m ll tu a r viewed by Kipnis (105) and P t and Bagdad (138) Inte e t h m inly been focu ed on th a ly pha of th sec etion of in ulin as a r sult f th hypothesis b ing put forward that a d c e ed and delay d a ly insulin pons to gluc stimul tion m ght be th ba p thogenic fa to in th development of all tag f th di betic ynd om in lu ding th p ediabeti stat (35) Th r i no g n al g em nt, h w v r on thi hypothe i (94 141, 159) Th f quently small s ri of highly l ted subj t d ff n in m th d of glu o timulation and techniqu f valuation of th insulin tory spon m ght well be fa to pons bl fo thi confusion

Th high p ed sp ition f d bete in th g ne al population e p cially in women (11) cl ly indicate th t a m thod of ev aling thi p ed po ition would be f on d bl impo tan in th a ly diagn nd p event on of th di In 1968 when th p e nt tudy wa ta ted n information w available about th di tribution f th a ly in ulin e pon in th g n ral popul tion Pr liminary data ha c ntly b n ported how v from a population tudy of m n in Upp al Swed n (85)

Th main purpo of thi pa t of th pr ent in tigation w to tudy th f quen y d tribution of th glu timulated ly insulin pon a fl ted by an in as in insulin on ntr ti n in th p ph al blood n a andom ampl f women aged 50 and to eval at to wh t xtent fa to such as glu o timulati n and l tive body w ght influen th magnitud of th pon In th f llowing hapt

study (117) none had a k value < 1.00. When retesting a subgroup five years later, 7 per cent had developed a k value < 1.00, however (118). Obviously that factors discussed above concerning differences in composition of studied groups and in test procedure might explain these divergent results.

The results of the present study indicate that a considerable number of women aged [illegible]0 have a glucose disappearance rate which is generally accepted to indicate a diabetic state. The significance of a low k value and of such factors as obesity, dietary habits and family history of diabetes for the future development of manifest diabetes needs a prospective study to be determined.

SUMMARY

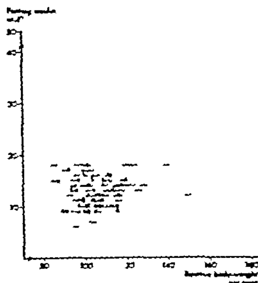
The present cross-sectional study of women aged [illegible]0 showed a wide range in glucose disappearance rate with a mean k value of 1.88 and a median k value of 1.[illegible]. The continuous and unimodal distribution of the k values did not permit to establish a limit between a decreased and a normal k value except arbitrarily. Using traditional limits a considerable number of women showed a k value which is generally accepted to indicate a diabetic state. Six per cent had a k value < 0.90, 11 per cent a k value < 1.00 and 17 per cent a k value < 1.10. A weak association was found between obesity and a decreased k value. The variance in relative body weight together with the variance in glucose [illegible] could, however, only explain 2 per cent of the total variance in the k value.

P[illegible] [illegible] monograph. Arab [illegible] [illegible] Lit., pp 115 - 121

T bl I S rum sul n n nt t n b f and ft int v n u gl o lo d in th t tal m t al of 352 wom ged 50 and in wom bg ouped o d g t l t body w ight

R latı body w ght (%)	n	F tı g			4 min			6 m			8 min		
					In ul n (mU/l) [1]								
76 170	352	13 9	5 3	3 47	86 7	46 8	1 390	72 6	45 6	12 495	60 9	37 4	14 380
< 90	34	11 9	3 8	5 19	82 9	37 3	20 185	66 5	30 3	20 137	53 4	4 4	16 11
91 100	77	12 4	4 0	3 2	72 5	31 1	2 38	58 8	25 1	1 180	47 5	20 0	14 136
101 110	106	13 8	5 7	6 47	92 2	55 1	12 390	77 5	57 5	14 495	64 7	45 1	14 380
111 1 0	70	14 7	5 3	7 36	93 1	43	8 45	77 5	38 5	27 230	64 8	33 9	23 195
121 130	31	15 1	5 0	7 30	84 8	46 9	26 184	76 5	47 2	0 18	66 8	1 7	22 180
≥ 131	34	16 7	6	7 31	93 9	58 2	29 345	80 7	58 1	27 340	74 1	45 2	38 260
F_b			5 06			4			2 25			3 78	
P			< 0 01			0 05			< 0 05			< 0 01	

1) M an S D and Rang



F g 2 Relationship between r lati e body w ight and fasting in ulin con entration in 352 women aged 50

In ul n e t on

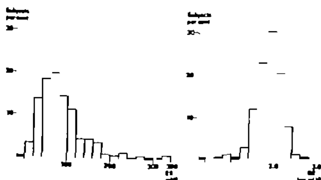
Th int avenou gl o lo d l ted an in rea in th rum in ulin oncent ation in all but one woman during th f t 8 min t aft r the tart of th glu o inj tion. A on d abl ang w s found in insulin on ent ation ached d ring th pe iod tud ed in th t tal mat rial a w ll at diff ent lev ls of latv body w ght (Tabl 1) Ob w men ched a gnif antly high r in ulin n ent tion than did l an women when t ted by on w y analy of var an Wh n th t t wa pplied to the in a in insulin on ent tion from th b al l l aft 4 6 and 8 minut th in ement d d not g nif antly ir s w th in re ing latv body weight xcept aft 8 m n t F_b fo th 8 minut in em nt 3 05 $p < 0.05$) Th p ak in ulin n ent at on w noted at 4 min te in 86 pe ent and at 6 min t n 11 p nt of th women. A peak in a below 10 mU l w noted n 4 p nt (n 8) and abov 200 mU/l n 1 pe cent (n 4 o th wom n.

E ly n l pon (ER)

In t p e t t g u n th in rem ntal in ulin c n nt ti n aft r 4 6 an 8 m n t w ed to al ulat th total arly in ulin

spon (ER) acco ding to Th ll t al (162) ER w cl ely o lated to oth m asu f th insulin in fo exampl th inc ea at 4 minut s (0 95) in as at 8 minut (0 99) and th a a b low th in ulin curv abov ba lin f om 0 to 8 minut (0 99) In th f llowing wo k up only th ER v bl will b tak n into ount

Th ang in ER wa cons d abl (1 559 mU/l) with a m an valu ± S D f 85 4 ± 60 4 mU/l and a median valu of 72 mU/l Th f qu ncy di tributi n wa skewed in th total mat rial with th l p toward high valu but t nded to be n rmal fte logar thmi t an f rm tion (F g 3) Th wa no indicat n of bimodal d tri buti n f th ER vari bl Th di tributi n wa th am wh n th m t rial w tri ted to wom n with a l tiv body w ght ≤ 120 p nt within ± 10 p nt of d al w ght' Th d entil of th ER di tributi n ar p nted in Tabl II. Only 2 p ent (n 8) f th w m n h d an ER b low 10 mU/l



F g 3 P entag di tributi n f th ly in ulin pon (ER) and th log thm in 352 w m n aged 50

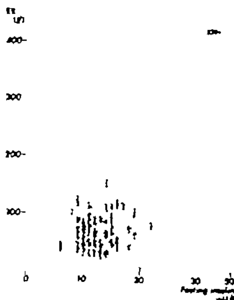
Table II D centil of early insulin r spons (ER) in 35 women aged 50

Decentile	ER(mU/l)	D centile	ER(mU/l)
1	30	6	83
2	41	7	98
3	50	8	115
4	63	9	154
5	72		

The mean ER f 85 4 mU/l in th total mat al may be compared w th a m an ER of 91 1 mU/l in a group of wom n with a r lative body w ght of 95 105 p nt and a body w ight of 60 0 64 9 kg (n 47) thu hom gen us as eg de total gl os and gluco dose p kg fat f w ght.

Facto nflu n ng th ea ly n ulin sponse

A w ak co latı n (r 0 26) was found betw n fa ting in ul n n nt ation and ER (4 97 p < 0 001)(F g 4)



F g 4 R latı nsh p betw n fasting insulin conc ntration and a ly ins l n spons (ER) in 352 women aged 50

The increase in blood glucose concentration during the first minutes of the test varied markedly as discussed in chapter III. The correlation between the glucose stimulation, irrespective of mode of expression and ER were however extremely weak (Table III). The weak correlation is illustrated in a scatter diagram where one of the variables of glucose stimulation, G_{stim} is plotted against ER (Fig. 5). Women with a G_{stim} below the first decentile of the G_{stim} variable had an ER which did not significantly deviate from ER in women with a G_{stim} above the highest decentile [79.7 ± 45.0 mU/l and 92.0 ± 60.0 mU/l (mean ± S.D.) respectively, t = 0.97, $p > 0.1$]. The correlation between glucose stimulation and ER was studied in the previously mentioned group of women with a relative body weight of 95–105 percent and a body weight of 60.0–64.9 kg (n = 47). Neither in this group of women was any significant correlation found between G_{inc} and ER (r = 0.05; t = 0.33, $p > 0.1$) or between G_{stim} and ER (r = 0.05, t = 0.33, $p > 0.1$).

Table III. Coefficient of correlation in 352 women aged 50. ER = early insulin response, G_{dose} = total glucose dose, G_{inc} = mean of increment in blood glucose at 4 and 6 minutes, G_{stim} = mean of increment per minute during the first five minutes.

Variable	ER r	t
G_{dose}	0.10	1.87
G_{inc}	0.12	2.25*
G_{stim}	0.08	1.50

*$p < 0.05$

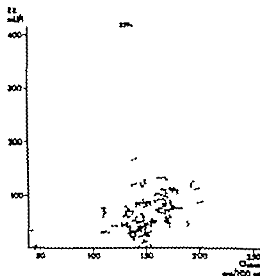


Fig 5 R lationship betw en glucose timulation (G_{stim}) and early insulin espon (ER) in 352 women aged 50

A w ak co r lation (0 14) wa found between th lativ body w ight and ER (2 63 p < 0 01) (F g 6) Furth rmor th m an ER in a ed statisti ally gn f ant w th inc ea ing lat v body w ght in women g ouped a co ding to lativ body w ght t st d by on w y analy i of va an (Tabl IV) Th var an in ER w on d abl in th total mat rial as well as in wom n g oup d a o ding to lat v body w ght w th low r va ian n l an and high va an in obe women. No dep nd nce was found b tw n elat v body w ght and ER when th h quar t st wa pplied to th total mat ial (Tabl V) A h gh f qu n y of ER abov th ght d ent l of th a a abl in th total mat al wa how v noted in wom n w th a elat v body w ght abov 100 p nt ompa ed to lean wom n (3 2 p <0 001) Th f equen s of women b low th f st and cond d ntil w not gn f antly diff ent b tw n th a g oups of wom n (x 1 4 p >0 05 and x 1 1 p >0 1 sp ti ly)



Fig 6 Relationship betw en r l tiv body weight and a ly in ulin spon (ER) in 352 women aged 50

Tabl IV Ea ly in ulin spon (ER) in 352 women aged 50 subg ouped o ding to lati body weight

Rel tive body w ight (%)	n	ER(mU/l) M an	S D	Rang
≤ 90	34	77 9	42 8	1 172
91 100	77	65 9	35 4	6 228
101 110	106	92, 2	72 3	1 559
111 120	70	91 3	54 1	22 280
121 130	31	90 1	68 7	7 271
≥ 131	34	99 3	75 0	9 416

F_{ob} 2 57

P < 0 05

Table V Women (%) in th total mate ial subgrouped a cording to r lat v body weight and early in ulin re p ns (ER)

Relat ve body w ght %	n	D c ntiles of ER I	II		VIII	IX
≤ 90	34	8 8	14 7	61 8	5 9	8 8
91 100	77	15 6	7 8	68 8	6 5	1 3
101 110	106	7 5	13 2	55 7	9 4	14 2
111 120	70	8 6	8 6	58 5	14 3	10 0
121 130	31	12 9	9 7	48 4	12 9	16 1
≥ 131	34	8 8	2 9	64 8	8 8	14 7
χ^2			22 3			
p			> 0 1			

A tepw mult pl g on analys wa p formed to timate th f a ti n of total va an in ER wh h wa du to th fa tor G_{do} G_{in} G_{tim} and latı body w ght Th f a t on wa tim t d to be 3 p nt

DISCUSSION

F a t ng n l n n nt t on

Th d t but on f fa ting nsulin on nt at o wa sk wed in th total m t al w th th lop tow d h gh on t at n wh h on t nt w th p v s po t (19 39 167) Obes wom n m nly ont but d to th kew d di t but on Th s in ag m nt with th no mal d t but on of fa ting n ul n on nt ation found n g oup nt in ng only ubj t w th body w ght of ± 10 p nt f d al we ght (73)

D p t a m jo ty of w m n of normal w ght o only mod r t d g of obe ty an so at o although w ak was found b tw n fasting l t t o and d g f obe ty Th n ag m nt w th lt bta n d n t d on s a of mo obe pat nt as vi w d by R b n w t (140) Oth g oup h f led to how

this s lation how v as view d by Ol f ky t al (128) The f ting concent ation of insulin i con id ed to depend n fa to asso iated with th deg of in ulin i tanc in p riphe al ti su (69 89 128 140) Th adipose ti u ha b n discussed mo t f quently in thi r sp ct. Ti u s oth than th adipose tissu fo ampl th mu le hav also b en on id ed a th ou c of th insulin sistan howeve Th w ak o relati n b twe n fa ting in ulin ncent ation and lativ body w ight in wom n in th p sent tudy may indicat that ti su oth than th adipo ti u may b th main d t rminant of th on entrati n of fa ting insulin in l an and mod at ly be w m n

Insulin s tion

The p ent inv tigation wa und tak n to tudy the a ly pha of th glu o timulated in ulin esp n Gluco on f many ec tag gu pabl of stimul ting th β ll to t insulin as viewed by M yhew t al. (120) Kipni (106) and Mal i (119) Studi s in man hav indicated that th glu o timulated el a of insulin biph i with an ea ly ap d phas followed by a late l w and su tained pha (30 139 165) Th biphas eto y spon ha als b en hown in studi on th i lated pe fu ed at pan s (24 46 71 90) indi ating th exi ten f pa t fun tional pools f insulin w thin th pan ti l t. Th fi t f th pool i con id ed to be a t ed pool of lim ted iz of adily v ilabl insulin Th a ly ap d pha of th insul n tion wa timated to ac ount fo only 2 p ent f th total ext tabl pan ati in ulin nt nt in th at in vitro (46)

If on on d th d lay involv d in th transp t of glu and in ulin, th evid n to sugg t that th arly el of insulin i alm t in tantaneou Th in ulin d l v y te d a p dly aft th fi t l and v ry l ttl in ulin will be l a ed aft 4 5 minut f om thi po l (17 46 71 162 165) Th lat pha of th insulin s c tion s ons d ed to tart within a few minut f th ta t f th glu o timulati n (17 46)

Insulin n ent ti n ma k dly high r in portal than in p riph al enou blood when m a u d imultaneously indicating a high emoval f insulin by th l v eviewed by F ld (66) Th lation betw en insulin on ent tion m u d at th l cations indicat s

however, that the insulin concentration in peripheral blood reflects the secretion from the pancreas and may be used to evaluate the insulin secretory capacity (17, 31).

In the present study the peak insulin concentration was noted at 4 minutes in 86 per cent of the women, which is in accordance with other studies (147, 148, 151, 152, 162). In studies in which the insulin concentration was measured even earlier than 4 minutes a few subjects had reached peak levels even before that time (123, 162).

Early insulin response

Many methods have been devised to express the magnitude of the early phase of the insulin secretion as reflected by an insulin concentration increase in peripheral blood. The disappearance rate of insulin from peripheral blood is higher than previously expected. The half life (T½) has more recently been calculated to be 3-7 minutes (30, 153, 160, 162, 165). In the present study the early insulin response (ER) was calculated according to Thorell et al. (162) using insulin levels at 0, 4, 6 and 8 minutes and a T½ of insulin of 7 minutes. This method also takes into account the part of released insulin which disappeared in the course of the test. ER will thus represent the increase in concentration of insulin that would have occurred if all insulin released into the general circulation had remained within the plasma pool (162).

A close correlation was however noted between ER and the incremental insulin concentrations at 4 and 8 minutes as well as the area below the insulin curve above baseline between 0 and 8 minutes respectively. This indicates that results obtained with methods using incremental peak values (165), mean values (139, 149), values at specific points of time (1, 50, 141, 147, 148, 152) and areas below the insulin curve above the baseline (143) may be regarded as comparable if the blood samples have been taken during the first few minutes of the test with the first blood sample not later than 4-6 minutes after the start of the glucose injection. When the first sample has been taken at 10 minutes or later (30, 94) errors may have been introduced in the estimation as far as the early phase of the insulin secretion is concerned.

Th p e ent c o s sectional study of women ag d 50 howed a wide rang ln a ly insulin r spon indicating ons d abl diff renc s in insulin sec tion capacity Th pr duc bility of th n u lin r pone to an intravenou glu os infu ion wa inv t g t d by C asl and Luft (33) They tat d that th v riation b twe n p ated t t we e urpri ingly mall A high p odu bil ty of th arly insulin spons has als been found by Hed trand and Bobe g (84) No rep ated t st w r pe formed in th p ent inv stigation A tanda dized te t p edu e including p tr atment with a ca bohyd at rich di t was used in ord r to tanda d th xpe im ntal on ditions a fa a po sibl

Th f equency di t ibution was kewed with th lop toward highe valu s but ontinuou and unimodal Th s ons tent with ult obtained in men aged 50 (85) a w ll as in a g up f y ung individual (159) The unimodal d st buti n of th ER v ri bl parallells th ondition f und wh n tudy ng o al (25 83) a w ll int venous glucose tol an (117 166 III) Thu no limit ould be tabli h d betw n a low' and a no mal a ly in ul n r spons except rbitra ily Th unimodal d t bution f th ER iabl and th ssumed high diabeti p d pos tion (II) do not pport th hyp th sis (35) that subj ts with a gen ti ally d t rmin d p ed spo tion f di b t would be charact ed by a ma kedly l w a l ulin r spon than subj ct without th p di po tion Th s hypoth will b furth di cu ed in sub qu nt chapt s

Th m an ER in th pr sent study wa 85 4 mU/l Du to d panci betw n th sults of in ulin a ay f om diff nt l bo ato rie (43) t would be ha a dous to mpa b lut valu from th p ent tudy with ult f om th tudi It may b m nti ned howev r that th m an ER in m n ged 50 in Upp al Swed n, wa about 70 mU/l (85)

An analy wa p formed to find out wh th f ting insulin on ent ti n glu o timul tion o b s ty w m j d t rminant f th magn tud f th ly in ulin spon Stud on insulin tion du ing o al glu o e tol an t ts hav indi ted an a so iation betw n fa ting insulin on nt ati n and th l t pha of th insulin pon Thi a oc ation w d d to be d p ndent on the be ty facto (8) It wa th n ugge ted that th a ly insulin

espon hould al o b expre ed a a r lat ve change f om th ba al lev l (9) A ve y w ak co r lation wa found b tween th fas ting in ulin on ent at on and ER in the p sent tudy The variation n fa ting nsulin con ntrati n could only xplain about eight p r nt of the total variance in ER No ignif cant co relation b tw n thes two va ables wa report d by Ste ky and Tho ll (159) An r or may b nt odu ed when compa son of a ly in ulin respon s a e p r formed in ubj cts of normal weight o mod rat d gr e of ob s ty if th early pon e is xpr ssed as pe c nt of fa ting in ulin oncent ration a has b n don in ome p ev ous tudi (9 166)

A p cial approach to the stimation of th magnitud of th arly insulin *espon e* has been advo at d by Ce s *and Luft* (30) *They* u d a apid injection of glucos followed by a con tant infu ion. Th fi t ampl was taken 10 minut s afte th ta t of th glu o inj c tion W th analogu computation of the data they judged th insulin *espon e in elat on to the blood glucose oncentration* assuming the T ½ of insulin to be 7 minut An insulin/gl o e atio has been us d to cal ul te th a ly pon e in oth studi *also* (3 147) Inh nt in th m thod th assumption that th magn tud of the a ly in ul n *pons* s lat d to th d g *of glu o timulati n* Stud e on th lationsh p betw n con ent at ons of gluco and in ul n du ing o l glu o tole anc t t b v nd at d that a lat on sh p x st betw n gl os timulation and th lat pha of th insul n *spon* (38 41 152) C as t al (38) *also found a co* lat on betw n th m gn tud of an int a nous glu load and th in *as of insul n con nt ation aft t n minut* Oth stud hav h wev ndi ated th t a kno r do spon lationship b tw *n glu o timulat on and a ly n ul n p n* only exi t at dos of gl o below f v to ten g ams (110 149) At high r glucose dos *no f th in as n a ly nsulin pons* was app rent. Simp on t al (151) po ted that an in r as of th glucos dos *from 25 to 40 g* d d ot n s th ly *sponse* Tho ll t al (162) d d not find any lat n b tw n th glu o timulation and a *ly e pons*

In th p t t dy th gl o do wa adju ted to body w ght, wh ch m an do ang *of* 24 56 g ams Th gluco timulati n a ed ma kedly g p f women w th dja nt lat e body

w ight and body w ight (III) Th glu os stimulat on ould only explain about on p r c nt of th t tal varian in th ER v riabl An rror may b intr duced wh n compar sons of a ly insulin pons s ar p rformed in l an o moderat ly obes ind vidual if the early r pons i exp e s d in r lation t glu o timulation

It i gen ally acc pted that ob s ind viduals hav an ugmented and su tained secretion f insulin afte an o al gluco load ind ti g an a so iation betw n obe ity and th lat pha e of th nsulin pon e a viewed by Karam t al (103) and K pni (105) In studi s con rning the a ly ph s of th n ulin spons th a s c ation has not be n evident (12 65 85 97 147 151 157) Usually a ma ked v ian e h s be n found in a ly sp n in obe subj t ind cating that som obe e indiv dual hav augm nted whil oth hav no mal o subnormal r pon Using g oup compa son t t in tho oft n small le of pati nts th in a in a ly p n wa how v r u ually not ignificant. M st of the wom n in th pr sent study w of n rmal w ght o only mode at ly ob Only about two p cent had a body w ight x ding d al w ight by 50 pe ent r m r In th total m te l a w ak o ati n was f und betw en lativ body w ight and th ea ly pon m inly d p n ding on a slight ov r p esentat n f high ER in m d at ly obe o women Th wom n with low ER w v nly d tr buted in th la of r lativ body w ght. The high va ian in ER in mod at ly obe women ompa d to l an women s thus con tent with sult btain d in p eviou studi in mo ob ubj ts

A deg of be ty total glu dos and bl od glucos l v l ch d a va iabl s whi h in th p nt study t ongly int lat d a tepwi e multipl g ion analys w p f rmed to stimat the ompound ff ct of th e v able n th ER var able Th analysi indi at d that the m gnitud of a ly insulin p n wa mainly d t rmined by fa to oth r than th glu o timulation and the d g of obe ity th s v abl togeth ould nly xplain about thr p ent of th total va an in th ER variabl Th significan f th magnitud f th ly insulin pon fo th futu dev lopment of manif t diabet will be di cu ed in sub quent chapte s

SUMMARY

A w ak a oc ation was found betw n ob ity and an incr ased fa ting insul n onc nt ation in lean and m d at ly obe women aged 50 randomly l cted from th g n ral population. Th variance in fa ting insulin lev l could only b xplain d by the obesity variabl to the extent of 10 p r nt nd cating that tissue othe than the adipo ti su a of major importance f r the fasting insulin conc ntration.

An int av nous glucos load el cit d a rapid incr as in insulin con ntration n almost all women with peak valu at 4 minut s in th major ty of as D ff nt methods u d to timate th magnitud of th ea ly in ul n pon may b con id r d comparable when th f t blood ample fo nsulin analys i tak n within 4 6 m nut s aft th start of th glucos lo d E ror may b introduced when th s pons s expr sed in r lation to fasting nsulin conc nt ation or d g of gl o timulati n a h ved aft r glucos loads gene ally us d n linical practi A w ak asso at on wa found b tw n obe ity and an augmented a ly insulin spon

A w d ange in a ly n ulin spon was found Th f qu n y d st but on w ontinuou and unimodal indicating that t s impo ible to epa at a g oup of ind v duals w th a low pons f om a g oup with no mal pon ex ept a bit ily Th unimodal d t bution of th ER a bl do not upport th hypoth s s that ind v dual with d abet pred spo t n m ght b ha a t s d by a magn tud of arly insul n spon that ma k dly d at f om oth ind v duals

Roman num al t to th h pte of thi monog aph. A b c num al f to th i n l at pp 115 121

EARLY INSULIN RESPONSE D RELATION TO INTRA VENOUS GLUCOSE TOLERANCE IN A POPULATION SAMPLE OF WOMEN AGED 50

Gö an Blohmé

Studi on insulin cr tory capa ity in man as eflected by an In s in insulin con nt ation in p riph al blood hav indicat d an a ciation betw en th magnitud of th ea ly in ulin pons and gl s di appearanc ate aft an intrav nous glucos load, as f t ob rved by Samols and Marks (144) and sub qu ntly by oth s (4 22 33 109 110 115 159) Th arly insulin r sponses wa sugg sted to be important in d t rmining th rat of glucose decay (144) Con quently a subnormal ea ly insulin spon ha been epo ted in subj ts with a n rmal fa ting blood glu o con entration but an im pai ed glu o tol an (4 32 147 151) Oth studie hav failed to how thi p tt rn of insulin s c tion, howev (3 94 141) Small s ri of patients cla sified a having an impaired glucos toleran a co ding to diff ent criteria and t ted with diff ent p ocedu might well be e ponsibl fo om of th d v gent sult ported.

Th main purpo of thi part of th p nt inv tigation w s to study th a ciati n betwe n th arly insulin espon and th glu co di appea an at with pecial ef enc to women with a low glu o di appea an e ate

F mate ial and m thod hapt I

RESULTS

Th varian in k valu wa ma ked t all l v ls of ea ly in ulin spon (ER) (Fig 1) Th co l tion b tw en ER and th k values w howev r tatistically signif cant in th t tal mat rial (0 40 7 91 p <0 001) a well a in women with a k valu ≥ 1 00 (r 0 35 6 43 p < 0 001) Fo both variabl the e was a tend ncy to l g normal di tribution (III IV) U ing l g valu s a somewhat high el tion oeff ient wa found in th total material (0 48) Th

att r diag am did not disclo e any other mathematical fun tion b tween these two variables which could give a highe d gree of fit. When the women w subgrouped a cording to elativ body w ight l an women had th high st value whil moderately ob wom n had th lowe t (Table I) In wom n with a elativ body w ight of 95 105 p cent and a body w ght of 60 0 64 9 kg (n 47) a value of 0 47 wa found.

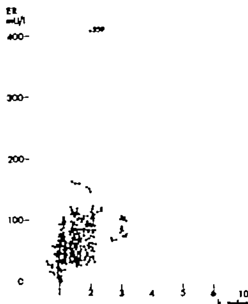
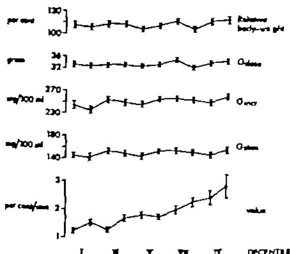


Fig 1 Relationship betwe n k valu and ea ly insulin spon (ER) in 352 women ag d 50

Tabl L Co lation eff c nts betw n arly in ulin spon (ER) and th k valu in 352 wom n subg ouped a co ding to elativ body w ght

R lativ body w ght (%)	n	
≤ 100	111	0 54
101 110	106	0 36
111 120	70	0 44
> 120	65	0 45

The co   lati n b tw   n ER and the k value   fu th   demonstrated in Fig 2 whe   women in th total mat rial a gr up d a co ding to th d c ntil s of ER The k valu vari d ma kedly in all group but did inc ea e with inc asing ER In ont ast th gluc   timulation emained almo t unchang d i r sp tiv of mod of exp   s on ju t a th   lativ body w ight did V ry w ak cor lation w e n equently found betw n th variabl G_{do} G_{in} G_{tim} and l tiv body w ight   sp ctiv ly and ER s discu s d in chapt IV



F g 2 R l t   body w ght G_{do} e G_{in} G_{tim} and k valu in 352 w m n ged 50 g oup d   o ding to d entil of a ly in ulin espon   (ER) M n ± S E

A tepw   mult pl   g   on analys   wa p form d in o d to timat the f   ti n of total va an   in th k alu which wa explained by th sum of va   bl   ER G_{do} G_{in} G_{tim} and lativ body w ight. Th   f a tion was found to be 19 p   nt.

Ju t   th va anc in k valu w   m k d at all l v l of ER th varian   in ER wa m ked at all l v l of th k valu Even in women with a k val in th low t part f th k valu d tributi n a wid ang of ER was p   nt (Tabl II III F g 3) Ten p   ent of wom n with a k valu < 1 00 h d an ER b l w 10 mU/l whil a few h d an ER even bov th m dian ER f th total m te al (72 mU/l) Women with a k valu < 1 00 w   bg ouped a c ding to elativ body w ght (T bl IV) Th m an of ER in th diff ent g oup did n t d viate gnif cantly from   ch oth   Th ang in ER in th s g up was w d   with th h gh t alu in mod   t ly b   women

Tabl II R l t body w ight f ating nsulin conc ntration and rly in ulin r pons (ER) n 352 w m n d n wom n ubg ouped co ding to th k valu

k alu %/min	n	R l t body w ght (%) [1]	Fa ting in ulin (mU/l) [1]	ER (mU/l) [1]	ER Rang
0 59 9 86	352	108 7 16 5	13 9 5 2	85 4 60 4	1 559
< 0 90	21	108 6 14 2	14 9 5 8	41 3 21 4	1 88
0 90 0 99	18	114 1 1 8	15 5 4 4	46 8 43 2	4 169
1 00 1 09	22	115 6 23 5	14 7 6 2	55 3 26 1	8 118
1 10 1 19	22	104 6 13 0	13 7 4 9	63 6 36 9	1 169
1 20 2 29 [2]	198	109 4 16 3	13 9 5 3	88 3 61 4	6 559
2 29 3 1 [3]	35	104 5 14 1	13 4 5 5	99 1 51 8	28 280
≥ 3 13	36	104 8 13 2	12 9 4 3	134 0 68 8	41 376
F_{obs}		2 00	0 76	10 31	
p		> 0 05	> 0 05	< 0 01	

[1] M an ± S D

[2] k v lu d centile XIII 2 293

[3] IX 3 126

T bl III Wom n (%) in th total m t ial by oup d ding to k val and a ly in ulin pon (ER) I th tati ti al analy i wom n w th ER val bo d entil II and b l w d ntil VIII w tak n t g th

k al %/m	n	D ntil of ER I	II	III	IV	V	VI	VII	VIII	IX
<0 90	21	33 3	9 5	28 6	14 3	9 5		4 8			
0 90 0 99	18	44 4	22 2	5 6	5 6		5 6	5 6		5 6	5 6
1 00 1 09	22	18 2	9 1	13 6	22 7	18 2	13 6			4 5	
1 10 1 19	22	4 5	18 2	31 8	4 5	9 1	4 5	13 6	4 5	4 5	4 5
1 20 2 29	198	7 1	10 1	7 6	11 6	9 6	13 1	10 6	10 6	11 1	8 6
2 29 3 12	35	5 7	5 7		11 4	8 6	5 7	17 1	22 9	8 6	14 3
>3 13	36		2 8		5 6	11 1	8 3	5 6	13 9	19 4	33 3

χ^2 86 2

p < 0 001

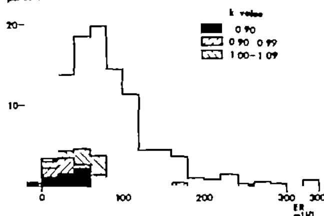


Fig 3 The distribution of women with low k values in the percentage distribution of the early insulin responses (ER) in the total material of 352 women aged 50

Table IV Early insulin response (ER) in women with a k value < 1 00 subgrouped according to relative body weight

Relative body weight %	n	ER mU/l Mean	S D	Range
≤ 100	12	44 2	35 7	1 120
101 110	9	46 8	49 8	4 169
111 120	9	39 2	16 0	22 64
> 120	9	45 1	24 8	7 88
F_{ob}		0 08		
P		> 0 1		

The unimodal distribution of the ER variable found in the total material (IV) was also present when the material was restricted to women with a k value ≥ 1 00

Th valu fo th second d centile in th di t ibution of ER in th total ampl wa 41 mU/l This valu coinc ded w th th m an ER of women with a k valu < 0 90 Wh n thi ER value wa chosen to s tabli h a limit between a low' and a n rmal insulin pons 17 p cent of wom n with a k valu ≥ 1 00 had a low' spon e The m an ER + one S D fo wom n with a k valu < 0 90 wa 63 mU/l As many as 41 pe cent of th t tal material and 35 p c nt of women with k values ≥ 1 00 h d an ER b low thi l vel

T ble V Som chara t ristic of wom n with an a ly insulin pons (ER) below 10 mU/l

Subj t	Body weight kg	Relativ body w ight %	Fa ting blood glu os mg/ 100 ml	k value %/min	G_{in} mg/ 100 ml	G_{tim} mg/ 100 ml
1	65 0	102	71	1 12	264	150
2	51 6	84	99	0 67	244	142
3	65 7	106	79	0 94	259	153
4	58 7	100	70	1 43	198	117
5	73 1	121	92	0 95	279	166
6	62.5	93	81	1 02	234	126
7	59 0	95	70	0 95	221	146
8	111 0	169	88	1 05	300	175
M an	68 3	108 8	81 3	1 02	249 9	146 9
S D	18 3	26 6	11 0	0 21	32 6	19 1
T tal mat al (n 352)						
M an	67 0	108 7	70 2	1 88	251 6	150 2
S D	11 2	16 5	8 3	1 10	37 9	29 1

Some characteristics of women with a very low ER (< 10 mU/l) are presented in Table V. Only one of these women had a completely normal k value. A significantly higher fasting blood glucose concentration was found in these women than in the remaining women ($t = 3.8$, $p < 0.001$) and three of them had a value exceeding the mean + two S.D. for the total material. They did not deviate from the remaining women as far as relative body weight ($t = 0.01$, $p > 0.1$) and glucose stimulation are concerned ($t = 0.1$, $p > 0.1$ and $t = 0.3$, $p > 0.1$ for G_{incr} and G_{stim} respectively).

Only one of eight women with an ER <10 mU/l had a close familial history of diabetes (sister) compared to 19.6 per cent in the total material. Three of seven women with children had given birth to a child with a birth weight ≥ 4.5 kg compared to 8.5 per cent in the total material. One woman had untreated hypertension and another was being treated for hypertension with chlorthalidone, which drug was withdrawn five days before the IVGTT.

DISCUSSION

A significant correlation was found between the early insulin response and the glucose disappearance rate in the present study, which is consistent with previously reported results (4, 22, 33, 109, 110, 115, 159). Although highly significant owing to the large number of women studied, the correlation found was relatively weak. The variance in insulin response could only explain 16 per cent of the variance in k value in the total material and 12 per cent in women with a k value ≥ 1.00. Subgrouped according to relative body weight the explained variance was highest in lean women. A weak association between obesity and a subnormal k value (III) and between obesity and an augmented early insulin response (IV) may be considered to be responsible for the weak correlation between ER and the k value in the obese groups. In a group of women comparable as to degree of obesity and total dose of glucose the explained variance in k value from the ER variable was 22 per cent.

The stepwise multiple regression analysis disclosed that about 80 per cent of the variance in k value in the present study must be ascribed to factors other than early insulin response, glucose stimulation and relative body weight.

Th rapid a ly pha of th insulin sec tion lasting for only a few minutes and the high disapp a anc rat of insulin from p riph al circulati n, as discus ed n chapt r IV indicat that nsulin r l sed during the arly phase has disappe red long b fo th period during which the glucose di app a anc at alculated Th in u lin circul ting at that tim thus originates from the late ph se of th insulin c tion Fu thermo th amount of insulin ted during th arly phase i very small It ha been timat d to a count fo only 2 pe cent of th total extra table pancr ati insulin ontent in th r t in vitro (46)

The gluco di app aran at may be a um d to depend not only on th irculating amount of insulin but l on facto s d t rmin ng insulin ensitivity in periphe al ti su a di cus ed by Alf rd t al (4) Evid n exist to sugg t that the w ll-documented as oc tion betw en th arly insulin respon and glu d s ppea an at i mainly indirect. Fa tors inh rited or acqui ed s a on equ nce of th evolution of th diabetic yndrom might well partly be und rlying cau s f thi as ociation

Impaired glucos tol anc

Th unimodal di tribution of th k value in th t tal mat rial (III) indi tes that a lim t b tw n normal and impaired glu o tole anc cannot b tabli hed except a bit arily A k valu below 1 00 ha f quently been judged as impai ed and k value below 0 90 a mar kedly impai ed, as di cus ed in chapt III. I spectiv of th val ch en fo definition, a wid rang in a ly insulin pon wa found in wom n with impai ed glu s tol an in th p nt tudy Th majo ity of th women had ER value in the low part of th ER-d tribution but only a f w of th m as low pati nt w th mani fe t diab te (VIII) Th m an ER in, f xampl wom n with k valu below 1 00 was about half that found in th emaining wom n. A few women with a low k valu had ER valu x eding th median fo th total m t rial how Th w d rang in arly in ulin pon en in wom n with impai ed nt v nous gl o tol ran consi tent with th eports (4 85) Th oc u en l an imp d glu o tol anc d sp t a no mal o n high ly in ulin pon furth support th umption that th a ation found betw n

th arly insulin response and th glucos disapp arance rate is mainly indi ct.

Low gluco disappea ance at despite a consid rable concentration of circulating in ulin indicates a stat of insulin sistanc in p ripheral tissu (89) Th hyp insulin mia s en du ing th late phas of an oral glucos tol anc t t in some patient with maturity ons t diab t s i onsid red to be s onda y to th ustained hyp gly em a and/or th oft n imultaneou ly occu ing ob sity as eview d by Kipnis (105) and Po te and Bagdad (138) An in as d ea ly insulin respons ha b en r po t d in a mall g oup of subj cts aft r marked w ight gain d p t a imultan ously occur ing d c se in the glu o di ppea anc ate which indicat that th ea ly insulin r pon also may be influ nced by the d g of in ulin sen itivity in p iph al ti su (89) Th w ak assoc ation b tw n obesity and an augm nted in ulin pon found in th total mat ial in the p esent tudy (IV) was not, how ver evid nt in women with k valu < 1 00 Th majo ity of women in th p ent tudy we e l an o only mod a t ly obes Th adipo ti u may not b th main d t rminant of th degr of in ulin n tivity in p ph al tissu in th p nt m te al

Subnormal ea ly n ulin pon

H althy indi idual with a low a ly insulin spon hav been supp d to hav a diab tic p dispo tion (35) No g n ally a cepted c it ria xist f d fin tion of a low pon e Th unimodal di t bu tion of th ER variabl in th p ent population sampl made it im p sible to tabli h th lim t ex ept a bit a ily (IV)

In th tudy by C as and Luft (32) th lim t b tween a low and a normal pon was ho n b t a ily u ng p ti nt with mild mani f t d abet o impai ed int avenou glu o tol nc as a f n group U ng a p c ally d gned glu o infu ion t t 23 of 28 diabe ti patient and 18 of 85 (18 p c nt) healthy indiv duals w re judged a low spond In th p nt tudy nly ght women (2 p c nt) had a low a ly insulin spons a pat nt w th mild or mod ate man f t d ab t (VIII) Of th s ght women nly on h d a compl t ly rmal k alu howev Th sult indi ate that a low e ly in ulin pon a s en in pati nt w th manif st diabet v y ld m o h althy ubj t w th normal gl cose tol an

Th dispa ate ults obtained in th pre nt study compa ed to th study by C asi and Luft (32) may b explained by diff en e in sel c tion of di betic patients and the diff rent m thods u ed for gluco timul tion d e timation of th magnitude of th a ly insulin r pon e as discu sed in hapt r IV

A l w arly in ulin re ponse was supposed to chara teris all stag of th diab tic syndrom (35) As a k val below 0 90 indi te markedly d c ea d glucos tolerance and h often been judged s diabetic (III) wom n with k val es below thi lim t m ght b u ed as a ef rence gro p fo th definition of a low spon As many s 20 p r cent of th total mat rial and 1 p c nt of women with a k al ≥ 1 00 had an a ly in ulin spon bel w the m an r spon in wom n with a k valu < 0 90 Using th m an pon + on S D f r delimitation th o sponding fr qu n es in a ed to 41 and 35 pe ent resp tiv ly

A dis u ed in chapt III a group of women w th low k valu may be regarded as h t og neous R p ated te t would p obably d limit a g oup of wom n with a mo constant d c e n th at of gluco e d cay Oth wom n would oc a nally hav b en cla fied a having pathologi al k valu c o ding to low d gr of disturbanc in the glu os hom o tas s o owing to m thodolog al ro s M v r f w d ta e avail bl on ning th long t rm gnif anc of a low k value fo th futu d v lopment of manif t diab te

A p o pectiv tudy m ght lu dat th lative signif an f low glu os d app an e at and a subnormal ly in ulin spon s fo th futur d elopment f manif t di b te

SUMMARY

Significant c lation w f und b tw th ly insulin po and th gl d pp an ate Th ER va abl ould nly explain 16 pe c t f th v an in th k al in th total m t al howev indi ating th t th a oc ation betw en th two r bl i mainly indi e t. A wid ang in ly ulin pons wa fou d in wom n with low k alu On av ag th s w men had bno mal ly in ulin pon Only 10 pe ent f wom n w th a k val below 1 00 had a low ly in ulin spo p tient with manif st

diabetes, however. The magnitude of the early insulin response was not dependent on the degree of obesity in women with low k values.

The continuous and unimodal distribution of the ER variable found in the total material was also found in women with a k value ≥ 1.00. No limit could be established between a normal and a subnormal ER except arbitrarily. Seventeen per cent of women with a k value ≥ 1.00 had early insulin responses below the mean ER for women with a k value below 0.90.

Eight women (2 per cent) in the total material had as low early insulin responses as patients with manifest diabetes. Only one of them had a completely normal k value. This indicates that as low early insulin responses as are seen in patients with manifest diabetes very seldom occur in healthy middle-aged women in the general population.

Roman numerals refer to the chapters of this monograph. Arabic numerals refer to the references listed at pp. 115–121.

Chapt r VI

INTRAVENOUS GLUCOSE TOLERANCE AND EARLY INSULIN RESPONSE IN WOMEN AGED 50 WITH A FAMILY HISTORY OF DIABETES

Gö an Blohmé

It i generally accepted th t h editary fact play an important ôl in th development of manif t diabet Th till con id r bl di g em nt about th mod of inhe tan how v (7 8 125 142 155) Ir pective of th mod of inh r tan a mo p o nounced p edispo ition for diabetes m ght b xpect d in wom with than in wom n without a family h story of the dis P evious tudi s have not indicat d an a ociation between family hi tory of diabet and impai d glucos toleran howe as evi wed by Wahlbe g (166) Th period before the dev lopment of impai d gluco tol ranc i called the p edi b ti pe od (51) It ha been gg ted that h althy subj t with a g n ti p edispo it n f diabete will b cha act ri ed by a a bnormal a ly in ulin spons (35)

Th main pu po of th pa t of th p ent nve t gation wa to tudy th glucos disappea an te and th a ly in ulin espon in women ag d 50 with a family h sto y of d abet

For mate al m thod and d f n t ons hapt I

RESULTS

Glucose d pp a n at

Th m an k valu and th f quen y f wom n w th a k alu b low 1 00 in wom n w th diab ti moth fath o bling w re not gnif antly low than n th (n g p of wom n without a family h to y of di bete (T bl I) N d d wom n with a family hi tory of di b t in th latt hav low k al than th refe nce g up Th ang in k val wa w d in wom n with a family h st y f d bet (F g 1) On woman ported diabet in both pa nt H k al w s 1 49 Six wom po t d diabet s in on p ent and a bling Th m an k al in th g o p was 1 94 ± 0 69 (S D) wh ch wa not l w than in th f en g oup

Tabl 1 Relat ve body w ight k value and ea ly insulin esponse (ER) in 352 women subg o p d s o ding to fam ly h tory of diab t

Family h story of d b te	n	R lativ body w ight (%) 1)	k value (%/min) 1)	k valu < 1 00 %	ER (mU/l) 1)	ER < 42 mU/l %
1 Moth r	39	110 8 20 0	1 76 1 06	10 2	69 5 39 5	30 8
2 F th	20	110 6 13 9	1 76 0 64	10 0	80 0 52 1	25 0
3 S bling	17	112 1 14 0	1 60 0 65	17 3	71 6 38 6	11 8
1 3	69 2)	110 4 17 4	1 71 0 92	13 0	72 0 45 0	27 5
4 Oth l t e	45	106 6 16 4	1 91 0 74		82 4 46 9	22 2
5	238	108 6 16 8	1 90 1 20	12 6	89 9 66 5	17 6
G o p 1 3 v rsu G oup 5	t/z	0 78	1 22	0 1	2 10	1 81
	P	> 0 1	> 0 1	> 0 1	< 0 025	< 0 05
G oup 4 v us G oup 5	t/z				0 72	0 73
	P				> 0 1	> 0 1

1) M an ± S D

2) Sev n women reported double h edity

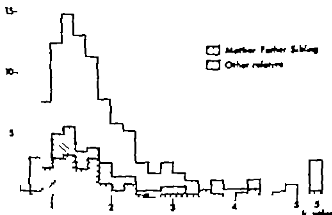


Fig 1 Th di tr bu tion of women with a family hi tory of dia bet s in th p centag dist bution of the k value in th total ma t al of 352 wom n aged 50

E rly in ulin e pon

A significantly low m an arly insulin pon (ER) and a s gni f cantly high f equency of ER valu s below 42 mU/l w f und n women with a cl s familial h story f di b t than in wom n without a family hi tory of th d e (Tabl I) Th o t n b tw en a clos familial h story and a subnormal ER main d wh n women with k valu b low 1 00 w x luded from th al ulat n (T bl II) In thi g oup of women with a k val ≥ 1 00 gnif antly high f quency f subnormal ER val s wa al found n wom n with a family history f diab t in oth lat v mp ed to the f r n gro p (T bl II) Th ang in ER wa marked in wom n w th family h to y f diabet s (Fig 2) Th woman with d ab t in both p nt had an ER f 83 mU/l wh h may b ompa ed w th med an ER of 72 mU/l in th total m te al Th six wom n who po ted d b tes in on pa ent and a bling h d m an ER of 77 5 ± 34 1 (S D) mU/l which w not ignifi anly low than n w m n in th f n g oup (t 0 45 p > 0 1)

T ble II R lat v body w ght k v l and arly nsul n r ponse (ER) in 313 w men with normal gluco tol an (k alu ≥ 1 00) subg oup d o ding to family hist y of diabetes

Fam ly hi to y of d b t		R lat body w ght (%) [1]	k l (%/m n) [1]	ER (mU/l) [1]	ER Rang	ER < 42 mU/l %
1 M th	35	109 1 18	1 86 1 08	73 7 39 3	23 172	34 3
F th	18	111 1 14 4	1 85 0 61	83 3 53 8	27 260	22 2
3 S bl ng	14	112 1 14 8	1 76 0 61	79 8 36 6	36 150	7 1
1 3	60[2]	109 4 16 5	1 83 0 91	77 4 44 1	23 260	23 3
4 Oth l r	45	106 6 16 4	1 91 0 74	82 4 46 9	24 244	22 2
5	208	108 3 16 2	2 08 1 21	96 2 67 1	1 559	1 5
G o p 1 3 r s	t/x	0 46	1 48	2,05		2 07
G oup 5	P	> 0 1	> 0 05	< 0 025		< 0 025
G oup 4 sus	t/x		0 91	1 31		1 69
G oup 5	P		> 0 1	> 0 05		< 0 05

1) M an ± S D

) S n wom n r ported double h dity

Fig 2 Th distr bution of women with a family hi tory of diabete in th per centage distribution of th a ly insulin r pons s (ER) in th total mate ial of 352 wom n aged 50

Obesity

Th mean relative body weight was not ignificantly highe in women with a family histo y of diabet s than in women in the r f r nce group (Tabl I II) The p valen of wom n with a elati e body w ight above 120 p cent wa how high among women wit a clo e familial histo y of diabet s (19 f 69) than in th f enc g oup (40 of 238)(1 99 p < 0 025) Th mean k value in th obes women with a clo e familial hi to y wa 1 53 ± 0 54 (S.D.) and m a ER 77 6 ± 46 7 (S D) which wa not gnifi antly lowe than in obes women in th fe enc gro p (1 85 ± 1 18 and 107 7 ± 81) f r U/l t 1 12 p > 0 1 and t 1 47 p > 0 05 spectively)

Pa ent ag s

Wom n with pa nts i whom on o both had died befo r the g of 70 did not hav ignif antly l w m ans of k-val s / ER a significantly high f q en y of k alu s < 1 00 a / ER < 44 m ompa ed to oth women (T bl III) N ith was th r m ar l body w ght g f antly h gh f wom n with a k-val / ER was Th m an ag of moth ed to 71 2 + 12 7 (S D) in th g o 10 1 (S D) omp f f th we 68 14 11 5 and 70 Th co ponding val

(t 1 0 p > 0 1) Th m an age of mothers of women with an ER < 42 mU/l was 75 1 ± 9 6 (S D) compared to 70 5 ± 12 9 (S D) in the remaining wom n The orre ponding value for fathers were 67 8 ± 14 4 and 70 9 ± 13 8 (t 1 64 p > 0 05)

Table III R lativ body weight k value and early in ulin r spons (ER) in 238 wom n without known family history of diabet s ubgrouped a co ding to ag s of the parents (at the tim of tudy or at d ath)

	n	Relative body w ight (%)[1]	k value (%/min)[1]	k value < 1 00 %	ER (mU/l)[1]	ER < 42 mU/l %
Both pa nt ≥ 70 y a s of age	100	108 3 17 4	1 84 1 18	11 0	85 6 56 5	18 0
On o both pa r nt < 70 yea s of ag	138	109 9 15 4	1 98 1 22	13 8	9 3 7 2	17 4
t/x		0 75		0 63		
p		> 0 1		0 1		

[1] M an ± S D

DISCUSSION

Gl o tol n e

I th p nt tudy no a so iation was found betw n a known famil y h to y of d b t s and dec ea ed tol an to an ntrav nous glu os lo d th g d ng m an k lu o f equ n y of w men w th k al b low 1 00 Th s o si t nt w th ault obta ned n oth r stud o gl o tol anc us ng intrav nou (11 126 137 166) as w ll o al gl o l ad as ew d by Wahlbe g (166)

A g oup of wom n w thout known family history of d abet s was used s f n g oup in th p nt tudy Som women m y have b en n d t l ly g t ga ding family h story of d abet as on o both p t w nknown o had d d at g s wh n th p val n of m l t d bet s low Unkn wn as s of manif t d b t s

s w ll as subclinical forms of diabetes among relative (discussed in chapte II) may hav furth r contributed to a h terogen ous ompo ition of th f renc group If th refer n e group wa tricted to women both of whos par nts had reached th ag of 70 no fu th information wa gain d onc rning th a sociation b tw n a family hi tory of diab tes and impai ed gluco tol an howev Fu th r m th m an ages of pa ent of wom n with a k alu < 1 00 w not significantly lower than in th emaining wom n Th sult indi cat that n xce of wom n with impai ed glu o tole an w c eened fal ly n gativ conc ning family h story of diabet owing to d ath of p r nts at arly ag wh ch m ght h on aled an associ tion b tw en impair d glucos tole an and a family h to y f diabet

The sults indicat that g n tic fa to s ppo ed to be invol ed in th evoluti n of th diabet c yndrom h not y t g v n any ff t on the tol an to an intravenou glu s l d in w m n ged 50 A th p val n e f manif st diab t a w ll as of impai ed gluco tol an e will inc a afte th ag of 50 an a ation m ght w ll ha been found if an old g g o p had b n tudied.

Ea ly nsul n re pons

Th p val n of a d ab ti p edisp ti n in women (II) will pr bably x ed th p val n f an impai ed gl tol an d fined a k valu < 1 00 found in wom n ged 50 (III) Th ndicat that even som wom n with a n rmal k val at th ag of 50 might w ll ha a p dispo tion fo th di A l w a ly nsulin spons ha b n ugg ted to be ch t ti f bject with su h a pr disposition (35)

In th p ent tudy a w ak tion w found betw en a cl familial hi to y of diabet s and a subn rmal ly nsulin pons Th con t nt with th subno mal spon p ted in th h althy off p ing of two parent w th di bet (143 148 151) and in bling f juvenil di bet (159) A on tant finding in th studi a w ll a in the pr sent tudy wa a wid ang in ea ly insulin pons in subject g n ti ally p edispo ed to diabet a w ll a in h althy c ntrol with ut family h to y of th di ea Furth rm r th only woman n th p sent study who epo ted di bet in both pa nts h d an ER abo th median valu f th total mat rial Thi finding

parallels results obtained by others (32, 143, 151). The mean ER in women who reported diabetes in one parent and a sibling in the present study was at the same level as in women with simple heredity. In addition, other studies have not revealed an association between a family history of diabetes and a low early insulin response (26, 33, 36, 114, 123).

The probably heterogeneous composition of the reference group in the present study has been discussed above. No furhter information was gained concerning the association between a family history of diabetes and subnormal early insulin response when the reference group was restricted to women with elder parents, however. The reference group might, however, conc al a considerable number of women with a genetically determined predisposition for diabetes. The association found may thus have been underestimated. The results indicate either that the gen (e) associated with the diabetic predisposition may influence the initial level of the early insulin response, as suggested by Cerasi and Luft (35), or that a decrease in this response has already started during the prediabetic period in some subjects with a diabetic predisposition who subsequently develop manifest diabetes.

A very high concordance rate with regard to diabetes has repeatedly been found in monozygotic twins, as reviewed by Harvald (82). A concordance rate approaching 100 per cent was assumed. Healthy members of monozygotic twins discordant regarding diabetes would thus be ideal for studies concerning a biochemical marker of the diabetic genotype. Studies in a few healthy members of pairs of monozygotic twins, the other member of which was diabetic, have given contradictory results concerning the early insulin response, however. In the study of Ce s a d Luft(34), two of six healthy members of pairs of twins discordant regarding diabetes had an early insulin response slightly below, and four above, the limit established to divide a low from a normal response. Completely normal early insulin responses have been reported more recently in other healthy monozygotic twins, the other members of which had diabetes (36, 49, 159). A recent study in monozygotic twins has found as low concordance rates as 50 per cent in twins in whom diabetes developed before the age of 40 in the diabetic twin, while the concordance rate was almost 100 per cent when diabetes had developed after the age of 40 (161). It was assumed that

environmental factor we of majo importance for th de lopm nt of diabetes in discordant twin In the bo e discus d tudi on ly insulin respons in twin th diab tes d v l ped befor th age of 40 in the diabetic twin. The twins tud ed may th s be heterogeneo as to typ and deg ee of gen tic influenc on th d abeti pr dispos t on. R sult obtained in twin studi s concerning biochemi al ma k r fo th diabeti geno type mu t be judged w th c ution until fu the info mation is available on th mode(s) of inh r tance of th diabeti syndrom

An a sociation was found betw n a clos familial h story of d ab t s and obesity in the pres nt tudy It wid ly held th t obes ty s ass ciated with th ons t of manife t diabet s (52 101 164 168) The small numb r of ob wom n with a family h story of diabet s and th wid ang in early insulin esponse in these women as w ll as n b s women without a family history of di bet s do n t p rmit mor d tailed analys s of th a sociation between family hi to y of diabete obes ty and early insulin respons A prosp ctiv tudy may g f th information about the r lativ s gnifican of th fa to in th evolution of th diabetic synd om

SUMMARY

N as ociation was found b tw en a family histo y of diab t s nd gluco e disapp a an te indicating th t gen ti fa t r suppo ed to be involved in the evolution of th diabeti yndrom may not be of importance fo th dev l pm nt of impa ed tol anc to an intravenou glu o load in women aged 50

A w ak asso iation was found between a clo famil al h t ry of di bete and a subnormal ea ly insulin spon The sults ind c t ither that th gen (s) a soc ated with th diab tic p ed sp tion m y influenc th in tial lev l of th ly insulin r spon o th t the p n had d c a ed as a c n qu n e of th evoluti n of the diabeti yndrom

Roman nume al I to th chapt f th monog aph. A b c nume al ef to th fe n l t, pp 115 121

DIETARY HABITS AI D BODY COMPOSITION IN A POPULATIOI SAMPLE OF WOMEN AGED 50 WITH SPECIAL REFEREI CE TO DECREASED GLUCOSE TOLERANCE AND/OR SUBNORMAL EARLY INSULIN RESPONSE

Ragnhild A nd son Lenne and Gö an Blohmé

Ob sity often pre d th manifestation of maturity on t di bet (5. 101 164 168) In addition to th ex s of n gy intake in lation to expenditu e th qualitativ composition of the food may also b a factor which tog th r with supposed g n tic fa to will influ nce th on t of the manif t f rm of th diabeti yndrom (164 168) Di tary habits in west rn count i with a high p rc ntage of calori in the form of fat and fin d sugar hav b en sugge ted to be e pon bl fo th in a ing p evalen of manif t di b t s (28 40 175) Th sult of th I ra l H a t Study h v not confirm d th sugg stion con ning th ignificanc of th qualitative compo tion of th di t (102) Furth rmo e sho t t rm a bohyd at f eding ha b en found to imp ov glu o tol ance both in h althy individual and n ubj t with impaired glu o tol ance a discu d by Brun ll t l (21) And s n et al (6) and Ho ton t al (89)

A p od of glu o intoleranc by d fin tion ch mical or sub linical d ab t (51) consid d to p ced th ons t of th mani f t fo m of th d betic synd om One pu po e of thi part of the p nt inv stig tion wa to tudy wh th r women with a low gluco d pp a an t aft an int av nous glu o load diff red f om ardomly s l ted w m n in p ct of d tary habit or body compo s tion

A low e ly in lin spon ha b n ugg sted to chara t ri e indi d al w th a d b tic p dispos tion who still hav a normal glu cos tol an (35) Few data re avail bl concerning th influenc of diff ent d ts on th a ly insulin r spon P liminary data hav indi at d th t w ght maintaining d t with a high carbohydrat ont nt wa f llow d by an in a in th ea ly insulin r spon (109) whil a gim dep v d f d ta y ca bohyd at s w follow d by a d r a d pon (47) A i th obj t of thi pa t of th pr nt in tigation wa to tudy wheth women with a low a ly in ulin

spons with o without a o_ ommi_ I w g d a p ara e rate dif ed from ra_domly l cted w m _ L ec c ny habits r body compositio_

MATERIAL

A random ample o wom n a d born m m day _ mo th wa select d for the pr _ study O_ w m n w k va ≥1.00 and a ea ly insulin r spon (ER) ≥ 4 mU l w n l ec (n 41) and acted a a r ence g p O th 3 particip ec tudy (Table I) Only 26 o 39 wom n in th p pulat tudy with an impaired gluco tol ranc d med a a k val < 1 00 (III w in stigated (g oup A g up B) W om _ 0 in pop lation tudy with a k val e ≥ 1 00 a d a bnormal ly ul pon defined a an ER < 4 mU l (IR tituted g p C (T b I) Th ER valu 41 mU/l r p nted th nd c entil th ER distribution in the population tudy and in ced with th m an ER in wom n with a k value <0 90

Table I I umb of wom n tudied on ning di tary hi tory in g oup A B C and R in p opo ti n to th t tal number with sp ti c t a in th popul tion tudy

		ER mU l	
		< 42	≥ 42
k value %/min.	< 1 00	12/21 G p A	14 18 G oup B
	≥ 1 00	27/50 G up C	36/41 G oup R

N n part pat ng w m

D ta on non parti ip ting w m in th p im y xamination and/ th IVGTT ha b n p ted a l (14 I) Body-w ght and l tiv body w ght d termin d t th tim f th IVGTT f wom n who did not p rti pat in th d ta y hi t ry tudy p nted in

DIETARY HABITS AND BODY COMPOSITION IN A POPULATION SAMPLE OF WOMEN AGED 50 WITH SPECIAL REFERENCE TO DECREASED GLUCOSE TOLERANCE AND/OR SUBNORMAL EARLY INSULIN RESPONSE

Ragnhild Arvid son L nn r and Gö an Blohmé

Ob sity oft n p c de th manif tation of m tu ity-on t di bet (52 101 164 168) In addition to th x ss of ne gy intak in lation to xpenditu th qualitative composition of the food m y al o be a facto whi h tog th with ppos d gen tic f ctor will influ nc th ons t of the man f t form of th diab tic syndrom (164 168) Di tary h bit in we t n ount ie with a high p rc ntag of calo i in th form of f t and fined ug have be n sugg t d to be sponsibl f the incr a ing p eval nc of manif st diab t s (28 40 175) Th s lt of th Is a l H a t Study have not confi m d the sugg tion con rning th ignificanc of the qu litativ ompo ition of th di t (102) Fu thermore hort t rm ca bohydrate f ding ha b n found to mp ov glu os t l rance both in h althy individu ls and in subj ct with impa d glucos tol ranc a discu ed by Br ns ll t l (21) And rson t al (6) and H ton et al (89)

A pe od of gluco intol nc by d finition ch mical o sub clin al diab t (51) is onsid ed to pr ced th ons t of th mani f t f rm f th di b tic ynd om One purpos of thi part of th p s nt inv t gatio wa to tudy wh th wom n with a low glucos d pp anc ato ft r an intra nous gluco load diff d from a don ly l t d wom n n p ct of di tary hab t or body ompo it on

A low rly in lin pon ha b en sugg ted to cha act i ndi d al with a d b tic p d po tion who still hav a no mal glu cos t l an (35) F w data ar a ailabl conc rning th influenc of d ff nt d t on th a ly insul n respons Pr limin y data hav ndic t d that w ght maintaining di t with a high a bohydrat cont t wa f llow d by an in as in th a ly in ulin r spons (109) whil a egim n d p ed of d tary ca bohydrat s was follow d by a d c as d po (47) A f the obj t of this part of th pr s nt i tig tion w to tudy wh th r w men w th a low a ly in ulin

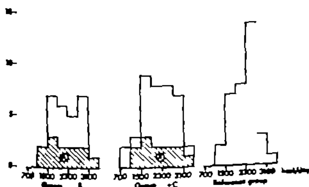
RESULTS

M l f qu ncy

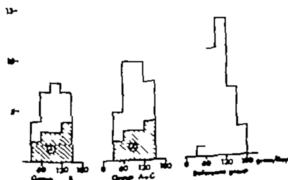
Th numbe of full meals p day vari d b tw n 1 and 3 in women in all gro ps with m an of 1 5 1 8 Sna ks such s off e with bre d and butt va ed b tw n 1 and 6 p day with means bout 3 pe d y in all group

Food consumption

Food n rgy fat and a bohyd t Th onsumpt on of food en rgy fat and total CHO howed a w d ang in all g oup (Fig 1 2 3) No sign ficant diff r n we noted b tw n th groups in th means of th s nutri nt t ted by on w y analys of varian (Tabl III) About 13 5 p r nt of th n gy wa pplied f om p ot in 43 5 p c nt f om fat and 43 pe ent f om CHO in th f an g oup



F g 1 Th di tr bution of f od n gy/day in m te al of women aged 50



F g 2 Th di tr bution f food f t/day in m t ial of wom n aged 50

Tabl IV Cont nt f nut nt p 1000 k l n di t of women ag d 50 n not signifi ant.
F t 2 7

		P t in		F t		Carbohydrates									
						Total		I Su ro		II La t		III Other sugar		I+II+III	
		g[1)]		g[1)]		g[1)]		g[1)]		g[1)]		g[1)]		g[1)]	
A	1	38 8	6 3	44 4	6 0	102	15	16	6	12	7	11	9	39	11
B	14	37 2	4 9	42 2	6 9	110	12	19	9	10	4	16	11	45	1
C	7	36 8	6 8	43 3	6 6	105	1	19	11	11	5	11	8	41	10
R f nc group	36	33 4	4 9	46 6	6	105	15	2	10	9	6	11	9	4	12
F_{ob}		3 75		2 30		0 79		1 31		1 16		1 20		0 67	
p		< 0 05		n s		n s		n.s		n s		n s		n s	

1) M n ± S D

T bl V C nsumptio f oth ential n t i t in m t ial f wom n g d 50 n n t ignifi t
F t 2 72

		Ca mg[1)]		F mg[1)]		V tamin A [1)]		Thiamin n g[1)]		Riboflavin mg[1)]		Nia id [)] mg[1)]		A corbic acid mg[1)]	
A	12	1210	630	14	3	4150	1670	1 7	0 5	2 1	0 9	13 1	4	9	40
B	14	1220	370	16	4	5010	1950	1 7	0 6	0	0 5	14 3	3 0	151	104
C	27	11 0	380	16	5	4900	2070	1 6	0 5	1	0 6	13 5	6	96	40
R f en g oup	36	1040	320	15	3	4720	1720	1 5	0 4	1 9	0 5	12 5	3 4	97	55
F_b		0 98		0 97		0 57		0 88		0 70		1 18		3 35	
p		n								n		n,		< 0 05	

1) M an ± S D

2) Pr form d

A trong co r lation wa obtained in th total mat rial (n 89) betwe n body fat calculated by the isotop dilution m thod and r lative body w ight calculated a p c nt of id al weight (r 0 92) (Fig 5) When computed within the g oup A + B C and R the corr lation remained very strong (0 93 0 89 and 0 93 re pectiv ly)

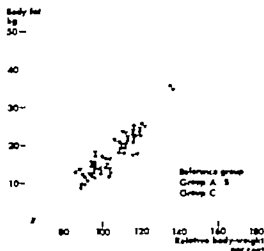


Fig 5 R lationship betw n lativ body w ight and body fat in a mat ial of wom n aged 50

W ight edu t on

Eight 29 and 26 p ent spe ti ly of wom n in g oup A B and C tated that th y h d t ied to r du e th body w ght p iodi al ly o cont ously du g th last few y ars ompa ed to 28 p c nt of th f en g oup (X^2 2 0 p >0 1) Th m thod used fo w ght edu tion w e num ous f om total ta vation during on o ev al d y a w k to ystem t exclusi n of sw t and f t from th di t Th r sult of th ex tion w howev r poor as the majo ty of w m n po t d unchang d or sl ghtly in s d body w ght. S v n t en 29 and 7 p t pe t ly of wom n in g oup A B and C po ted a w ght ga n of mo than 5 kg du ing th l t 5 y om pa ed to 28 p nt n th ef nc g o p (X^2 4 7 p >0 1) Th v st majo ty of th wom n had a w ght gain betw n 5 and 10 kg D ta of w ight g n w al o obta ned fo wom n l ss f d as on part cipati g in th pr nt d ta y h t y study N o g if ant d f f n e in th frequen y f wom n with a w ght ga n of mor than 5 kg wa found wh n p ti pat ng and n n p t pating women w r analyzed tog th (X^2 6 6 p >0 05)

DISCUSSION

A di tary hi to y i a o gh method for tro p ct analy s of th mean f od intak dur ng a e tain p d of tim (48) Many ro s may be introduc d s h as faulty memory and diff ult in coop a tion. The techniqu u ed in the p e t study was a fully wo ked out and information obtained was ch ck d and o s h ck d Chang in body w ight we discuss d and p al nformat on wa coll t d nc rning subgroup of the di ta y ca bohyd at On a d th sam specially t ained nut it nl t di titian p f rm d all interview Healthy middle g d wom n may b xpected to oop ate w ll n such an interview In p t of th s fa ou abl ndition only group compari on and eg e ion analy s w pe f rm d

En rgy supply and n gy b la

Th stimat d m an calo on umpti n w con t nt with th theo tical qui em nt of n gy in middl aged wom n with l ght to mod at activity alcul ted a o ding t FAO/WHO (2010 2230 k al/ 8 4 9 3 MJ) (61) Anoth la g sub ampl f th p nt population in tig t d by I aks on (93) u ing a omewhat d ff nt t chn qu fo di tary history show d a m an al on umpti n which ag ed ery w ll with th finding of th p nt tudy In an ea l popul tion tudy of wom n in Got bo g Swed n (76) th m an n gy intak in a group of wom n aged 50 w al ul t d to be pproximately 1500 k al Th latt tudy w how pe formed with a d ff ent m thod (24 hou all) wh ch n d d to g low val s (93 173)

N s gnificant d f n x ted n gy pply b tw en th ub g oup in th p nt n tiga on No w th any d ff en in body ompo tion hang body w ght d ing th la t f v y ar Th ult di th t f cto th than b ty and th balan betw en gy pply and en gy xpend tu aus an im p d glu o t l n and/o bnormal ly in l pon n m ddl g d wom

In lini al situ ti an y m thod n ded f al lat n of th d g of b ty Th t ong l ti n b tw body f t, m a u ed w th th sotop d l t hn qu and l t body we ght alculated from a h ght w ght tabl d te th t th l tt m thod suff t mo t l al tu t n f m ddl ged l an o mod a ely obe wom n o n d

Qualitati e compo ition of th diet

Ref rence g oup Th d tary habit among wom n in the refe n e group ha e b n compared w th a p viou report on food con sumption n Sweden (171) Wh n calculat d p r unit of energy the nut ient cont nt in the food of women in the present study was about the sam a in th av rag Swedish daily food consumption Th con umpt on of ucros wa howev r only about half of th m an uc ose onsumpt on al ulated for th total Sw di h population Thi finding i onsistent with th re ult ported by Yudkin (176) who found a x and g elat d intak of ucros Th statistics reported by Ab amson (2) from an invest gat on p form d in 11 Europ an countri s by Euratom al o onfirm d an g r lated d c a in consumption of su ro Th l ulat d intak of v tamins and min rals wa con ist nt with p ev eports in Sweden (171) and may b judged as s ff c ent co ding to th no ms suggest d by Eeg Lars n t al (57)

In w t n nt a st ong a ociation ha been repo ted betw n consumpt on f f t and u (174) In th p esent study thi a s i t on wa w k wh h may b explained by th sex and ag d p d nt d ff n d ta y habits The food energy suppli d wa mo t dep nd nt on th amount of fat on umed

Wom w th mp d glu o s tol ran Wom n studied w th k l < 1 00 onsumed sl ghtly diff ent food quality than th f g o p w th a g f tly high r protein onc nt ation th d t Th d ff n n on nt ation of fat total ca bohydra t d o d d t h gn f ant l l A sel t on for wom n w th o m l body w ght and p obably mo nt est in m intaining a b l d d t w bta ned n g o p A Th th num i lly mall d ff od q l ty fou d b tw n women w th an mp ir d gl o tol n d th i n g oup m y n pa t b att ibut d t an o o l t In dd t o no gn f ant orr lation w obta n d b tw th gl t t and th k al in th f n g p

Th lt d t th t th qu l t t ompo it n of th di t t i n jo n p t th d lopm nt of a low glu o di pp t n d l d wom n Th sumpt on d not l d how th po l ty th t th q al tat nd/or quant ta t mpo t t th d t m y u ot s d abl mportan in th

futu   developm nt of th diab tic syndr m   to the manif   t fo m of the di   ase W ight gain in wom n with l w gluco   d sapp a an ate may b   suggested to be   f sp c al importan   for th   d   elopm nt of manifest diabet s

Th ordinary mean   a bohydrat intake found in th di ta y history in estigation in the pres nt tudy was 200   250 g p r day and nly a few women repo t d a ca bohydrat   ntak below 150 g pe   d y   A di t low in carbohydrat s has been found to decr a   th tol   ance to glu os   as discussed by Brunzell   t al (21) and Ander on   t al (6) Th   a bohydr te f   ding du ing th ee day   b f   th IVGTT w s c nsidered to h v   r sto ed a d   r ased glucos   tole ance which m y hav   b   n found in women on a low carbohydrate di t   a   fo   xampl   du ing a w ight   educing r gimen.

Wom n with subno m l   a ly   nsul n   ponse No significant diffe enc   was found   n food qual ty betw en wom n with a k valu ≥ 1 00 and a subno m l   a ly insulin   spon   d fined as in the pr sent chapte   and women in th   f   en   g   up   Th   r sult indicat   that subnormal   a ly insul n   espons   in m ddl   ag d w m n do   not d p nd on deviati n f om   rdinary di tary habit   Ext em diets might, how v   a   e chang   in th   ly   ulin   spon just as   s d s   ibed for th lat   ph   f th   insulin espon   s r viewed by Mala   (119) P   limina y   epo t   h ve indi ated th t a w ight maintain   g di t d p   ved   f   a bohyd at s m ght   u   a d cr a ed   a ly in ulin   spons   whil   c   bohyd at   rich di t m ght cau   an in r   ed   pon   (47 109) In dd tion weak but ignif cant co r lation w   obtained betw en t tal   on umption of en   gy fat and s c o   sp   tiv ly   and ER in th   f   en   group i   th p esent   tudy An in   ased   rly in ulin   pons   h   b   n   po ted aft r a w   ght ga   du   to   d   t w th   h gh   ntent   f fat d spite a imultaneously o ~u   ing d   in gl   os   tole an   (89) Th in   a   wa th   ght to em   g   from th   dec   in n ulin   ns t vity in p   ph   al ti   ated w th th   st t   f ob   ty and not f om th   fat con   ntrati n in th   di t pe   A   s   t n alth   gh w ak w   found betw   n be   ty and an a gment d   a ly insulin r   po   in th   total m t   al of th   p   ent popul tion   tudy (IV)

F   th   tud   a   eded to l   d t th   l ti   ff   t and b ol g   al   g   f an   f q ant tat v   and q al tativ   hang   in d   ta y habit   on th   ly in ul   spo

SUMMARY

A arefully worked out t hnique for tudying di tary histo y wa used to in e tigate di tary habits in a mat ial of women ag d 50 Th *mean calorie consumption wa 2260 kcal in a andomly s l cted group* of th wom n About 13 5 p r cent of th en rgy was supplied from p ot in 43 5 p r c nt f om fat and 43 p cent from CHO in this g oup The intak of suc os was about h lf of th m an c nsumption calculated for th total population p obably dep nding on a d r asing intak of u rose with age

Wom n with k alu s below 1 00 and/o subnormal ea ly insulin re pon s did not diff from th randomly l ted wom n in r p ct of ne gy supply body ompo it on or ec nt chang s n body weight. *Num ri ally mall diffe n c n th* qual tat ve composition of the di t with a l ght b t s gnifi antly h gh p ot in conc nt at on in th di t of women w th low k alu compa ed to th andomly l ct d wom n may n pa t b att but d to an r o of a l t on Th sults nd c t th t ord nary d ta y h b t in Sw d n a e not a majo d t r m nant of th d v lopm nt of a low gl os d sapp a ance rate or a subnormal ly ul n sp n n m ddl ag d women

R man un l t th h pt of th monog ph Arab c m al t l t pp 115 1 1

Ch pt VIII

FARLY INSULIN RESPONSE IN MATURITY ONSET DIABETES MELLITUS

Gö an Blohmé and Johan Wald n t öm

On of th m in purp f th p nt population tudy was to d t rmin th p evalenc of low a ly in ulin r sp ns in th g ne al population Th a n fo this wa th hyp th is th t a low ly in ulin p ns might char t individuals with g n ti lly d t rmined p edisposition fo diab te (35) No accepted c it rion xi ts by which to tabli h a limit b tw n a low nd a normal pon In the study by C asi and Luft (32) p tient w th mild m tu ity onset diab te impai ed glu o tol anc w u ed a f enc group A few th tud have b n p formed on small numbe of p tients with manif t di bets (18 50 95 123 144 147 151 165) No o ve y low ea ly in ulin ponse wa port d in th majo ty of th e p tient Du to d epan betw en th sults f in ulin as y f om diff rent labo atori (43) data f m th tudi s annot b us d fo dentif cati n of 'low ly insulin r pond s n th p ent population tudy

Th main purpo f thi p t of th p nt inv tigati n wa to tudy th a ly insulin espon i m ddl aged p ti nts with m ld o m de at m tu ity-on t di b te u ing th am t h qu f gluco timul tion and f stimation of th magnitud of th ly n ulin pon a u d in th populati n tudy

MATERIAL

M ddl aged mild mod at matu ity n t d beti 18 wom and 16 men, we lected f m an outp t ent clinic f d b ti s in Got bo g Sw d n u ing c t ri mention d in hapt I Th m g w 52 8 and th ang 40 6 y a Th patients h d be n f ed to th cl du ing th p vious few y a for f th inv s tigati n of glu osur a with o without clini al ymptoms of manife t diab t g th st l s of w ght and tir dn Th m an p iod b tw n if tion of th d agnosi of diab t and th tudy wa 1 6 y with ange f m nth to 6 y Th majo ty f

pat nt had already b en advi d b fo e th fir t isit to th clinic to r d ce th ir consumption of carbohyd ates During th ob ervation at th clinic all pat nt had once or r p atedly had glucosuri of vary ng d g ee and a fa ting blood glucos c nc ntration exce ding 120 mg/100 ml A cla ificat on of th patient according to th initial fa ting blood gl os c ncent at on giv n in Tabl I

Tabl I In t al fa t g bl od gluco e conc ntration in 34 diabet pati nt

mg/100 ml	n
< 120	1
120 149	5
150 199	14
≥ 00	14

Th n l p t t w th n t al fast ng bl od glu o concent a t on cl f d n T bl I below 120 mg 100 ml after a p iod of xcl of w t omp n nt f om th d t followed by d creasing gl o nd w ght loss of 4 kg wa ubs qu ntly found to ha e a g l od gl o t at on of 140 mg/100 ml

o th p t t had b t at d w th insulin. E ght p tient t t d w th t lb tam d o hlo p opam d fo a few w ks th Th th py w int pt d in two pat nts 3 month and p t t 10 0 mo th b fo th s tudy Since the first vi it all p t nt had b n p b d an o d nary di t fo d a l d g t ti n of al s fats and arbohyd at A w t 16 4 ± 4 9 kg (a g 0 18 kg) occu ed in e th Th h d oft al dy sta t d b fo e th f r t vla t t l Th t ted m rmal body w ght n th y a b fo th bet w 8 4 ± 1 7 kg (ang 61 136 kg) Th act al w u ± 14 1 kg (ng 57 111 kg) and r l ti body l t w 0 p nt (g 9 181 p c nt) D t w ight and w bo t p ods of lfonylu a th apy b d d th fa t blood gl o o nt ation d d g of th m j ty f th p t ent A few pat nt w th newly

detected d s ase still had a marked f sting hyp rglycemia at th tir of the pr nt study (o r sults) Th di b tic pati nt reported a family history of di b t s in 68 pe c nt (Tabl II) a ording to cri teria in chapt I

Tabl II Pr val nc of known family h sto y of diabet s in 34 d ab tic pati nts

	n	%
Cl se fam lial history	17*	50 0
M th r	10	29 4
F th r	7	20 6
Sibling	5	14 7
Other r lativ	6	17 6

* Fou patient epo ted fam ly h to y in mo e than one of th subg o p

Two pati nts (ca A and a B) h ving ma kedly l viating r sults will be d s ibed in mo d ta l

Ca e A i a woman aged 57 H moth and mat rnal a nt h maturity o s t di bet s Sh h d n t uffe ed fr n liv r g ll bl or pan r ati di a Sh h d be n tr ted fo hyp t nsi will salu tic diur ti in th ly 1960 Gl uri w a first by the Te t tap m thod aft few weeks of this t in Mar h 19 A few day lat sh w s f ed t th lin II fasti g bl d gl c concent tion wa 160 mg 100 ml and h l cont lin l fin of glu os by th Cl n tix m thod An o al glucos t l r n t al was p formed (50 g of glu o lly ep t d aft 50 r l m s) bl blood glu s ent t w 125 230 and 307 mg/100 r l t a 30 and 60 minut p ti ely Sh w dvi d to r du n sumpti n of fat and bohyd t No d ug w r s ed A r d ti of 12 kg in h r body w ight w noted b eq ently and th l dy w igh light w ight g n (5 kg) w noted 65 kg co r ponding t l u at th tim of th P ent t dy w ent II f ti g blood gluc s t to body w ght of 107 p

has since th initial p iod of fasting hyp rglycemia b n normal and no gl cosuria ha be n noted At the pr s nt study h r fasting v ous blood glu o conc nt ation was 86 mg/100 ml and he k value 1 55 An oral glucos tol ranc t st p rform d as abo e 4 months after th int av nous gluco toleranc t st showed blood glucos con ntr tions 100 167 185 mg/100 ml at 0 30 nd 60 minute r sp ctiv ly

Cas B is a man aged 46 H r po ted no family history of diab t s H had not uff r d from liv r g ll bladde or pancr atic di a es Glucosuria was fou d at a h alth ontr l n D cemb 1970 H h d no symptoms of diab t An or l gl c s tol an t st was performed (ca A) showing blood glu os nc ntrations of 90 205 240 mg/100 ml at 0 30 and 60 minut s sp tively H wa th n f red to the clinic and on month lat he had a fa ting blood gluco e on c nt ation of 128 mg/100 ml and th ur n ontained 11 g glucose ov r 24 hou H wa advi d to d th consumpt on of fats and ca bohyd at s No drug w u ed A duction of 12 kg in hi body w ight was noted during half a y a H mainta n d thi low r w ight and at th t m of th p e nt study h s body w ght wa 84 kg cor s pond ng to a lat body w ight of 112 p c nt Ex ept for once (6 g/ 4 hou s) h u in h be n f from glu os nce th f r t adm s n. At th p nt tudy h f sting nou blood gluco on nt ation was 73 mg/100 ml and h s k valu 0 97 An oral gl cos tol anc t t w p f rmed as abov fou month aft the intrav nous glu o tol an t t showed blood gl cos on nt at o s of 84 166 and 198 mg/100 ml p ct ly

Fo m thod h pt I

RESULTS

Th ang at g bl d glu o on nt at on wa 71 328 mg/ 100 ml Th m an k l wa 0 74 ± 0 28 (S D) and th rang 1 55 0 1 p t m t (34)(F g 1) Two p t ent o of whom C B h d k l wh h m k dly d d th oth p t nt Two oth p t t h d k l oi 1 00 and 1 05 p ti ly Fasting hyp rgly m w l t d w th a low k lu (0 63 4 19 p < 0 001)

k value

1 5

1 0

0 5

100 150 200 250 300 350

Fasting blood glucose mg/100 ml

Fig 1 R l t nsh p b t w n fasti g blood gluco on nt ation and k valu n 34 pati nt w th diabet

Th fa ting rum insulin n ent ati n anged f m 8 to 40 mU/l with mean f 16 4 ± 6 4 (S D) mU/l A co lati n wa found betw n lativ body w ight and fa ting insulin conc nt ation (0 53 3 34 $p < 0\ 001$) Th incr a of in ul n on nt ati n du ing th f t ight minut aft the ta t f th gl o l ad w v ry l w or unmeasu abl in all but two of th pati nt (Ca A and B) (T bl III) ir spectiv f th fa ting insulin lev l (F g 2)

T bl III Conc nt at n of rum sulin and blood gl wh n f ting and aft an int v nou gl load in 34 d abeti p tient ER a ly in ulin p n

	D abeti patient n 32[1)]		C A		C B	
	Insulin mU/l[2)]	Blood glu o mg/100 ml[2)]	In ulin mU/l	Bl od glu mg/100 ml	In ul n mU/l	Blood glu mg/100 ml
0 min.	16 2 6 5	164 71	25	86	9	73
4 min.	17 6 7 4	443 95	61	369	82	444
6 min	15 1 5 8	415 87	48	332	69	371
8 min.	14 6 5 4	394 77	47	322	41	312
ER	1 6 5 3		40		69	

1) Ca A and C B a ounted fo p t ly

2) M an ± S D

DISCUSSION

The manif st form of th diab ti yndrom over a wid rang of disturbanc in the gluco e hom ostas Mo t patients sel ct d fo th pre nt tudy p nt a v ry mild form of th dis a Owing to di ta y restr ction and ubs qu nt r duct on in body w ight and in a few cas s hort p riod of ulfonylur a th py om of th pati nts tudi d had b om normals d a ga d fasting blood glucos o c nt at on a d in two cases al o a ga d tol anc to glucos in jected int a nously

A di t w th a high arbohyd at cont nt w p scrib d du ing thr e day befor th t t Ca bohydrat fe ding ha b n hown to imp ov th tol ran to glucose in subj ct w th mpair d glucos tol ran a d ussed n hapt r III A high r m an k valu and a h ghe f qu n y of k alu s of 0 90 1 00 was found than epo t d in oth t d on d ab t pat nts a v w d by Wahlb g (166) The ca bohyd ate f d ng may h e mp ov d th glu o e tol ranc in ome of th p t ents th p s nt t dy Th l t on of mild cas w how p bably th ma n u of thi d sc pancy

In ul t on

Th t no glu o lo d l t d a mea u able in r a in insul n nt t on ly h lf of th pat nt n th p s nt study Th p k n w y low n all but two pond s Th p ak l w h d t 4 m t wh h o ist nt w th r ults obtain d th pop l t t dy f o d bet wom n (Chapt IV) Thi d t th t th d ff betw m t ty n t d abet c and o m l t t o g th ly n l n pon s ma nly q t t

Th n d l n nt t t om ba I I l l t t l w n t d l t 8 m t a w r ft a b low th b l l l wh h d k t al of th a ly n l n pon (IR so t 0 p t o th p t t Ex pt f th two bj t w th IR d t nal all d b t p t t had FR b low 10 ml I IR w kl l t d t th k al wh h n l f nt w th t t j w th t n t t d abet iew d in h pt V l t h p gly m wa t d with d IR p t w th k d t t g hyp gly m no l p l d

Th ult in th pr ent study a con st nt with result f om oth tudie on maturity ons t diab ti showing a p onoun d hypo espon vity of insulin to an int av nous gl o e load pecially in subje t with fa ting hyp gly em (18 50 95 123 144 147 151 165) Studi in p tients with j enil d abet hav hown an ext m ly low o non m asu abl a ly (68 96 165) s w ll a lat (10 59 96 136) insulin pon ndic ting f th r d t o ti n f th th in lin c tion c pacity

Th in ulin cr ted d ri g an oral gl o tol an t t i n ide ed to o iginat mainly fr m th late ph of th insulin secr tion An inc a ed and stained in ulin spon aft an o al gl o l d w eported in th ea ly 1960 s in m tu ty n t d abetic tempo arily r ing the old n pt f an in ulin d f cien y a th majo def t in manif t di b t (172) Th hyp n ulinism ha how v mainly be n att b ted t th f equ nt imultan ously occu ring tate f obe ty (105) An ov r p entat o of b subj ts wa found in th diabet c group in th p nt tudy 38 p r nt having a lative body w ght x ding 120 p ent of d al w ght ompa d t 18 p cent in th population ampl Th a ociat n b tw en obe ty and an in ed fa ting in ul n l l f und in th di b ti g p i con tent w th ult btain d in non d b tic subj ts (IV)

N gnif ant o lati n w i und between lativ body w ight and ER in th g oup of patient h t g neo a oga da d g of di tu bed glu homeosta Th tw pati nt with positi val of ER d sp t f ting bl od gl o n nt ati ns l ghtly in e c of 120 mg/100 ml w how b s In th popul tion tudy w ak ti n w i und betw be ty and an a gm nted nsulin po (IV) Th sult ind t th t th d g of hyp gly mi i th main denom nato i th m gnitud of th a ly insul n pon in bj t w th man i t di b t

Two pati nts n th p t tudy h d a quant tati ly t tally diff rent a ly in ulin po w th an ER f 40 and 69 mU/l r sp cti ly val wh ch i ll j t bel w th nd and i fth d entil of th ER di t bution in th popul ti n tudy (IV) Th y had n rmal f ting blood gl on t ti and k al of 1 45 and 0 97 pe ti v ly Both h d m y p t th tudy b en f ed to th lin with gl and mod t fa ting hyp gly em A on id abl w ght edu tion h d p ded th emi on in th i

glucos homeostasis It may well be that th l ea ly in ulin spons s would have be n markedly lower if me su ed du ing the f ting hyper glyc mic p iod as non of th patients in the present study with fasting hyp rglycemia had an evid nt incr ase in th insulin concentration \ ight r duction of th same m gnitude or ev n g ate was not followed by normali ation of ER in oth r subj cts in th pr s nt study d pit normalization of th fa ting blood gluco concentration

A marked ncreas in th in ulin r pon after an oral glucos load was po ted in ob se diabeti s aft r a onsid rable w ight r duct on associated with normal zation of fasting blood glucose on cent tion and gluco e tol ranc (54) In two p ti nts with juv nil diab t studi d w th o al glucos lo ds du ing a pe iod of remis ion a d layed but augm nted spon has been r ported (87 98) In anoth pat ent th magn tud of th insulin respon e to an oral gluco e lo d chang d inv ly with the blood glucos conc ntration d ing th ours of ubsequent xac bation and impro em nt (108) A low in ulin r spons to an oral glu os load ha be n reported during p ods of em s on in a few oth patients with juvenile diab t (10 70 98) Th r sults indicat that a r mission in impaired glu os homeo ta may b followed by an improvem nt in the l t insul n pon Th r s lt of th p nt tudy indicat th t this imp o ment may also be al d fo th early insulin r pons An imp o ement n a ly insulin sponse ha furthermor be n reported tw p ti nt n em ssion from diabetic ketoacido is wh n test d w th t ous nj ctions of glucagon and tolbutamid (67)

F rth t d s on the n ul n s cret on c pacity a e n ed d in p t t d ng period of em on of th r gluco hom osta is to l d t th pot nti lly r ble natu of b ta c ll dysf nctio

SUMMARY

M ddl g d m ld o mod t matu ty on t d abetics we e ch t d by ry low o non m a rable a ly in ulin spons (ER < 10 mL l) Th p ti n w n more p onounced in pati nt w th f ting hyp gly m Two pat nt in r mis ion or p rtial rem ss on th d ang d glu os hom o tasis aft r a m k d w ght ed t n howed how a p ompt cr se in in lin con t t to th gl o l ad w th an a ly in lin r spon (FR) w th n th mal g a j dged f om th pop lat on tudy Th

sult indicate that an imp ovem nt may occ n th early insulin spon in some patients with d ab t during a p od of emiss on

Roman num al f to th ch pt r f thi monograph. Ar bic nume al f to th t nc l t, pp 115 121

Chapter IX

GENERAL DISCUSSION

The cross-sectional study of the present population sample of women aged 50 is intended to be the first phase of a long-term prospective study with the main purpose of evaluating whether a subnormal early insulin response will predict the subsequent development of manifest diabetes.

The age strata of 50 was chosen as a starting point for the present study as the probability of developing manifest diabetes in Sweden has been reported to be still rather low up to this age (72). Hence, for ages below 50 the average annual morbidity risk was estimated to be 0.4 per thousand while after 50 a markedly increased risk was found with age, especially in women. These results are consistent with the data collected by the National Center for Health Statistics in the U.S. (52).

The prevalence of manifest diabetes (1.3 per cent) found in the present study (II) is consistent with the general finding that 1-2 per cent of the total population in western countries has this stage of the disease (52, 62, 72). The well documented strong age dependency in the prevalence of manifest diabetes shows, however, that the average prevalence in the general population does not reflect the total morbidity risk for this stage of the disease. The total morbidity risk may be defined as the risk to an individual of developing manifest diabetes up to the age of 90. However, many of these presumptive diabetics will not develop the disease as they will die before the onset of the disease. A study of death certificates in Sweden indicated a total morbidity risk of 13 per cent in women and 6.5 per cent in men (7[illegible]). In that study correction was made for reasonable assumptions of probable gaps in the registration of diabetes and excess mortality among such persons.

These high prevalence figures agree well with the data collected by the National Center of Health Statistics in the U.S. (52). The women in the present study reported diabetes in 11.4 per cent of their mothers and 4.9 per cent of their fathers (II). When only parents who had reached the age of 75 were taken into account the frequencies were still higher, 15.5 and 7.0 per cent respectively. Reasonable [illegible] suggest that the real prevalence of diabetes predisposition is still higher (II). A prevalence of 20 per cent has been suggested (135, 155). In the present random sample of women aged 50 one [illegible] number of women might thus be expected to have

a diab tic predi position and conseq ently to b candidat for l t de el pm nt of manifest di b tes

Population studi have indicat d th t an impa ed t l an to an ral gl cos load is not uncommon n the gen l popul ti n sp ial ly in th ld ly (25 52 83) Th e f w long t rm follow p studi s nc rning the ignificanc of an impa ed gl os tol an f th f tu d elopment of th manif st form of th d s as (132 133 170) Availabl data indicat howev r th t th t of de lopm nt of mani fe t d b t in the futur s r lat d to th n tial deg of imp ed glucose toleranc Subj t with bo d lin valu m y have unchanged gl s tole anc fo a on d abl p r od of tim (29 133) or ev n bow an imp o ement (131 133)

Av il bl data onc ning th lo g t rm ign f an of an impai d tol rance to an int avenou gluco load a t ted to a few su vi v rs f my ardial infa ction followed up f p to 5 y ars (135 166) All wh d veloped manif t di b t had initially low k val

N ep ated t st w e pe f med n th p s nt populati ampl as th s women h d taken pa t in sev al xt n tudi and fu th r tudi s ar planned fo th f tu e It s w ll kn wn that th gl s disapp a anc at may a y from tim to tim n th sam individual as discussed in ch pter III A tanda di d t t pro ed in luding p treatment with a bohyd t rich di t w u d n th p ent tudy in ord r to standa di th xp im ntal condition far po sibl

Th di tribut on of th k val s w ontinuou and unimodal in th p sent c o s tional study wh h o tent with th epo ts (11 116 117 166) Th mad t impo bl to tabl h a limit b tween d cre sed and normal glu o tol an except b t ily Six p ent f th wom n had a k al < 0 90 11 p nt a k al < 1 00 and 17 p cent a k valu < 1 10 (III) Thu ing th t ditional d fini ti n fo a path log al k al on d abl part of women ag d 50 will h v k valu wh ch sually a pted as ep enting a chemi al o subclin al form of th d b ti ynd om Comp abl p ev lenc f gu have be n ep rt d in andomly el t d men ag d 50 in Upp ala Sweden (85) It em nabl to sum that women in th p nt tudy with markedly imp ed gl tol anc andida te for manif t d bet n th f tu

Th w d r ng n k alu s found n th pr s nt tudy was not du t th ar t on in d gr of ob lty or glucos stimul tion (III) A w k a oc t on was found how r b tw n obesity and a d cr as d gl cos d sapp ar nc rat wh ch I in agr m nt with oth r r ports (11 85 116 117 137) Wh n studying wom n with a k alu < 1 00 mor tos ly no s gn f ant d ff n was f und in body compos tion com par d to a randomly s l t d r f r nc group howe r (VII) This r ult indicat th t ob ty w ll not b th major det rminant for th d lopm nt of mp ed tol ran to glucos inject d intrav nously n m ddl ag d wom n As obe ty oft n pr c des th onset of m nl f t d ab t s (5 101 164 168) w ght ga n may b consid red to b a m j d t n nant fo th futur d elopm nt of man f st diab t s

D t y h b ts ha t equ ntly b n ons d red mportant for th d l pm nt of d ab t s (8 40 175) Th f nd ngs in chapt r VII d not ind t how that d tary h b ts r of imp rtanc for th d lopm nt of mpa r d gluco tol r n in m ddl aged wom n D t y hab ts m ght t g th w th ob ty b of ons d rabl impor t n to th futur d l pment of man f st d ab t s as w ll

A u n odal d t but on of blood glu o I l aft r a glucos l ad h oft n b n l n d t pro mult g n b s for th inh rit nc d b t St nb rg (155) and St nb g t al (156) h e dis uss d th hyp th s d d t th on lus on th t a continuous d tri b t w th n l n od d s not n s ar ly ndi at a mult g n c t nt ous ar bl D sp t an a sum d h gh dia t p d p t n n th p s nt popul tion sampl (II) th d strib t o th k lu w ont nuou and unimodal wh ch cons st nt w th h po t 11 116 117 166) Th nflu n of a g n or g n s w th th o t p ed po t on on th d l pm nt of a l w l a pp n t n s to b of m nor importan t l ast p t th a t o t n w found b tw n a fam ly h s t t nd b o mal k l (VI) Th s r sult on t nt w th o t 11 1 6 137 166) Th r lts nd at th t sub j t w tr ll d t n n d d b t pred spo t on w ll not b h n l w k alu omp d to subj t w th t t p p m bl to m th t l w glu o s ly d l ped a n q of s l wh th d g f n t ty to n l n

in p iph ral tissu s might be of m jo impo tanc Th g n ti influenc might contribute mor t ongly to th f rth dev lopment of the d abetic synd om

Th magnitude of the a ly insulin spons to an intrav nou glu co e load as fl ted by an incr in nsulin con nt ation n th p ph al blood is a biological va iabl gg sted to b f sp c al imp tanc f judging the susceptibil ty to diab t (35) Th hypoth i that a low arly in ulin re p n chara t sti of all tag of th diabetic yndrom in luding the p ediab tic state (35) ha howev been qu stion d a n rmal a ly insulin r spo s hav been epo ted en in patient with gl o intol ranc (3 94 141) and in th h althy member of pai s of monozyg t c twin th oth m mb of wh ch w diab tic (36 49 159) Fu thermor Simp on t al (151) hav d i bed as in wh ch manifest diab t d eloped twenty m nth fter m derately high arly insul n r pon e had be n ecorded.

Th no gen al ag ement h w best to stim t the ly in u lin spons A d cussed in chapt IV th ele s of in ul n in es pons to an intrav nou glu s lo d s o d d to be almo t in tan taneous and th insulin tion will dec s ap dly aft th first apid pha Furth rmo th d sapp an t of insulin from th p riphe al blood will be ath h gh w th half l fe of insulin of 3 7 minutes (30 153 160 162 165) Data indi at th t blood ample fo insulin analy s m st be tak n v y soo afte th gl l d with th fi t blood ample being tak not l t than 4 6 min t s aft th tart of th glu o inj t on Of ev al m thods judged to b ompa bl fo th timati n of th ly in ul n pon (IV) the m thod nt o du ed by Tho ell t al (162) w s ed in th p ent tudy Th m thod i ba ed on ep ted analy of th in ul nt ti du ng th fi st few minutes aft th glu load. Th alcul ted va bl f th ea ly in ulin spon (ER) sugg t d to ep ent th inc a e in in ulin on ent ation th t would ha ccu ed f all insulin elea ed into th g n al cul tion had emained w thin th plasm pool (162)

Th insulin pon w s l ted by a gl o do dju ted to body w ight n th p nt tudy D p t th wid ang n blood gl co con ent tion w found d ng th f t few minut of th te t (III) Thi w al evident t d ff t lev ls of lativ body w ight w ll in mo hom g neo gro p f women a eg rd body w ight and elati body weight. Th ults ind at s w d ang in th

olum of d stribution of glucos and agr e with pr vious r ports (32 9) o sign ficant corr lation was found b tw n the variab l s G_{tm} and ER and a v ry w ak but significant orr lation b t w n th ari bl s G_{incr} and ER in th pr sent study however (IV) p ss d n r lat on to th glucos stimulation as has been don in som prev ous studi s (3 32 147) The continuous distributi n of th ER ar abl d d not p rmit d finition of a limit between a "low" and a no mal arly nsul n r sponse xcept arbitrarily (IV) This is con st nt w th r sults obtained in oth r studi s (85 159) In th pr sent tudy a n th study of C rasi and Luft (32) patients with maturity n t d b t s w r us d as a r fer nc group A v ry low or non m asu abl arly insul n r spons was noted in th vast majority of th p ti nt a finding con ist nt with oth r reports (18 50 95 123 144 147 151 165) Of sp cial int r st is th obs rvation that two d b t p t nt w th remission n th ir di turbed glucos homeosta s lt m rked w ght r duction had an e rly insulin r spons judged t be w th n th no mal ang (VIII) It s r asonabl to assum that th ir rly nsul n pons would have be n m rkedly low r during th ir t ng hyp gly m p od indi ating that a r mi s on h d lso o d n th nsul n r t on c pacity Th hypoth sis that low ly n l n spon n during a hyp rglyc mic period may be bl iu th pported by th obs rvation by G nuth (67) of an mp m t th glu ag n and t lbutamid stimulat d arly insulin p n tu pat ts n m s on from diabetic k toacidosis n th pop l t n mpl ght women (2 per c nt) had an arly in i pun l w n pat ent w th manif st diab tes Only on h mpl t ly o mal k alu (1 43) (V) Th s data may be int r p d ll w F tly th s wom n may ha e pr viously h d m n an l t d b t and w r now n r m ssion. Remis ions d t urd g in th ly pha of man f st diabet s a F jan 4) S ondly th s wom n may b candid t s th l pm nt f m n f t d abet s in th n r futur Th r sults ar y th t low rly n ulin r spons s re s n in w th n t b t y ldom occur in h althy m ddl th al popul t on and annot b a ch ra t ist c ts of w m n w th a d b t c pred sposit n

Th hyp th sis that a subnormal arly insul n e pon will h ac t is all stages of the di b ti yndrom (35) m an that subj ts with a subclinical form of th di m ght b u ed a fe n g o p to stabli h a limit b tw en a 'low' and a n rmal spon Subj t with a normal fasting blood gluc s con nt atio and a k val below 0 90 ar usually onsid ed to h ve a subclinical form of di b t (III) In th p sent t dy wom n with a k alu below 0 90 h d n av g a low r ER than oth wom n (V) Although th ange of arly insulin s pons was con id abl in th wom n th m j ty of th m h d ER value within the low p t f th ER distribution Thi i con sist nt with othe r po t (4 85) Wh women with a k val b l w 0 90 w u ed a a f en group to d fin a low ly n ulin pon a on id rabl part of th total popul tion w judged a low r spond s how v r It obviou that group of wom n w th low k valu s is h ter g neou eg ding th in ulin ec tion p ty

An a ociation ha p atedly b n eport d betw en th ly in sulin spon e and th glu o di ppea an at (4 22 33 109 110 115 144 159 V) A di cu ed in chapt V thi ti ems mainly to b ndi t. Und lying f to m y b gg ted to influen both th ly insulin sp n and th glu o d pp a anc ate The main d ection of thi infl n n th v ri bl will be toward low value in s bjects who b equently d l p manif t dia b t An in s d ly insulin spon ha ntly be n po ted afte a w ight g in a soc ted w th d in gl s d pp anc t (89) In th p s nt tudy w ak but gnif cant lation w found betw en lat body w ght and ly insulin spon in the total material (IV) and betw en nsumption of alo fat and s s on th on hand and ly in ulin pon on th th in a ub ampl (VII) Ob ty and pec ally ec nt w ght g in oft n p ed th on t f manif t di b t (52 101 164 168) It vid nt that a l w glu o e di ppearan t m ght be ompan d fo h to o l g p riods by a n rmal a ly neul pon Th gnif ca of th ER l v l in wom n w th low k al f th f tu dev lopm nt of manif t d b t n ed p o p ti tudy to be d t rmin d

Th ER abl had a unimodal di t bution n th p ent study (IV) w ll in oth epo t (85 159) A h s b n di cu ed in onn tion with glu o tol an a unimodal di t bution do not n arily pro multig ni d t rmin ti n of a o tin ou v labl

GENERAL SUMMARY

Th pr nt study was p rformed on women aged 50 randomly el t d from the f mal population of thi ag In Goteborg Sw d n Th st dy w s ppl m nt d by an inv stigation of middl ag d pati t with mat r ty onset d abet s

Th pr v l n of m nif st diab t s in wom n g d 50 w s 1 3 p r nt wh l a onsid rably high pr v l nc was found in their pa ts Th p al nc of known man f st diab t s in th i moth r was 11 4 p r c nt and in th r fath rs 4 9 p c nt Wh n only pa nts who had ch d or pas ed th g of 75 w r tak n into a ount th figur w t ll h ghe 15 5 and 7 0 per nt sp ctiv ly P obabl u d r gi t tion of diab t s among p r nt and the f ct that a con id rabl p rt of th m w li ing at th tim of th study and thus still at r sk of dev lop g th dis a indicat that the diabetic pr di po ition m y b v y h gh in th population tudi d

Th tol ran to gluco inj ct d int av nou ly xpr ssed a a k alu p s nting the glucos d sapp arance at in p r c nt p r minu t sh wed a w d range in wom n aged 50 with a m an ± S D of 1 88 ± 1 10 nd med an valu of 1 59 Th d st bution of th k v l s was ont nuou and un mod l S x p cent of th wom n had a k valu < 0 90 11 p ent k lu < 1 00 and 17 p r t a k v lu < 1 10 A w ak n g t o lat on was f nd betw n elativ body w ight and th k l Th tudy ng wom n with a k alu < 1 00 m r clo ly th it nd t d how th t neith r obes ty no di ta y h b ts w i m p tan f th d l pm nt of a low k valu No associ tion wa f u d b tw i m ly h sto y of diab t s and a low k v lu indi ting th t h d t y f to thought to be ociat d with th diabet pr d sj tu d t m to b a majo d t rm nant of th d l pm nt of l w k l n m ddl aged wom n

Ih t o glu o load li t d a ap d l as of in lin a ll t d by n in ulin con t tio n th p iph al bl od n lm t ll w m w th a pe k on t ation with n 4 6 mi t s Th m g t d i th ly l n pon (ER) wa not lat d to th blood gl o u t h d d g th f t few m n t of th t t Th d t b t n th ER bl w o t uou and nimod l Con q ntly o l t uld b t bl h d betw n a low and ormal

pon x pt a bit a ily A significant cor lati n was found b tw n ER and th k valu Th ER va iabl xpl ined 16 p c nt of th vari an n th k valu A w ak association was found betw n ob sity and an augment d ER. W m n with a subnormal ER did not diff r from an domly s lected women in pe t of body mpo iti n o di ta y habit Wom n with a lo familial hi to y of di bet had light but igni fi antly l w ER than wom n without.

P ti nts with matu ity ns t d abete h d very low or non m su bl *arly insulin pon Th s wa p ially vid nt in p tient with* fa ting hypergly emi Two p tient in mi ion of th d turbed glu homeo tas had an bviou a ly in ulin p n aft a p od of w ight eduction. Thi ind at that a emi ion h d al o cu ed in th insulin ec tion pacity Eight women (2 p ent) in th popu lati n ample had a l w ER a th maj ity of the diabeti p t nt Only one of them had a ompl t ly normal k valu A low a ly insulin pon a ar en in diab tic p ti nt will c n eq ntly v ry s ldom be f und in healthy middl g d women and annot b a h a t i tic featur fo th m j rity f women with a diab ti p ed sp iti n Women with a k valu < 0 90 had on v g low ER than oth women but th ang in ER was wide nd a n d abl ov l pping xi ted b tw n wom n with l w and wom n with n rmal k valu

In onclusi n, th finding of th pr ent inv tig ti n supp t th hypoth th t th ly in ulin pons will d a d ing th ev l ti n of th d ab ti ynd om Th initial m gnitud of th ly insulin pon m y be g n ti ally d t rm ned a w ll Subj t with a ubn mal a ly *nsul n pon may c n q ntly on titut a h t og n* g up Som subj ct m y h v a subnormal pon n quen of th evolution of th di b ti ynd om wh oth m y hav a g n tically d t rmin d bnormal spon n t p c ted with di bet A l ng t m p osp ti tudy m ght d t min th l ti signif can of f to ch low k al s bnormal ly i sul n pon obes ty d etary h bits and family h t y of d b t fo th f tu d lopm nt of manif t di b t

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Supplementum 567

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A co-twin control study

By Mårten Myrhed

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ALCOHOL CONSUMPTION IN RELATION TO FACTORS ASSOCIATED WITH ISCHEMIC HEART DISEASE

A co-twin control study

By

MARTEN MYRHED

STOCKHOLM 1974

Tryckeri Balder AB Stockholm 1974

"En mångårig uppmärksamhet, riktad åt sjukdomar hvilka förekomma hos dem, som öfvermått begagna alkoholhaltiga drycker, har lemnat mig en vidsträckt erfarenhet, huru mägtiga dessa drycker äro, uti att undergräfva menniskans så väl kroppsliga som moraliska hälsa

Magnus Huss, M.D., 1849
Professor of Medicine, Karolinska Institute
Head of the Department of Medicine
Serafimerlasarettet

Many years' interest in diseases in persons inclined towards excessive consumption of spirituous liquors has provided me with wide experience of the power of drink to undermine both the physical and moral fibre of mankind.

Contents

Abbreviations and Terminology

BSA	Body surface area
ECG	Electrocardiography
IHD	Ischemic heart disease
IVGT	Intravenous glucose tolerance test
OGTT	Oral glucose tolerance test
SALP	Serum alkaline phosphatase
SGOT	Serum aspartate aminotransferase (formerly called serum glutamic oxaloacetic transaminase)
SGPT	Serum alanine aminotransferase (formerly called serum glutamic pyruvic transaminase)
SGT	Serum gammaglutamyl transpeptidase
SVEB	Supra ventricular ectopic beat
VEB	Ventricular ectopic beat
U	Unit
Concordance	The occurrence of a trait in both members of a twin pair
Discordance	The occurrence of a trait in only one member of a twin pair
MZ	Monozygotic: monozygosity. Twins originating from one fertilized ovum, thus identical in genetic make-up
DZ	Dizygotic; dizygosity. Twins originating from two separately fertilized ova, thus genetically related as two ordinary sibs
Pooled zygosity group	Monozygotic and dizygotic twins analysed together
HAG	High alcohol group (for definition see p. 14)
LAG	Low alcohol group (for definition see p. 14)
M	Mean
SD	Standard deviation

Introduction

Cardiovascular disease has long been the major cause of death in most Western countries accounting for approximately 50 % of the deaths in men 50 to 64 years of age. About two-thirds of these deaths are caused by IHD (Björck 1963 1968, Björck *et al* 1970 Stamler 1973 Vedin *et al* 1973) So epidemiologic research has concentrated on a search for different factors associated with cardiovascular diseases. Some risk factors (Simborg 1970 Fejfar 1972) are mainly genetic, others mainly environmental, but most are a combination of both. Smoking, which has become a major concern of modern medicine, appears to be influenced by genetic factors, although the habit may be regarded as an environmental trait linked to a genetic constitution (Fisher 1958 Friberg *et al* 1959 Cederlöf 1966, Liljefors 1970)

The present study concerns another of man's habits, a habit which is far older than smoking, the imbibing of alcoholic beverages. This habit has attracted the attention of both medical and public opinion over the centuries and has often been regarded as a threat to man's ability to live well. To date, many investigations have been made on alcohol consumption in relation to health. However most of these have concentrated on problems of etiology prevalence, care and social and psychiatric aspects of alcoholism, rather than morbidity and mortality among alcoholics, a subject not systematically investigated until recently Even then, somatic investigations were generally carried out on vagrant alcoholics, i e. subjects suffering from prolonged intoxication and pronounced social and physical deterioration. This implies that excessive drinkers in apparently adequate social circumstances and maintaining a normal life have been ignored. Furthermore, such research has been mainly into causes of death with the most obvious associations with alcohol ingestion, such as cirrhosis, cancer of the upper respiratory and digestive tracts, accidents and suicide. The increased mortality due to degenerative cardiovascular disease demonstrated in association with alcoholics has only recently been given the attention it deserves.



There are early references in the literature to the cardiovascular effects of alcohol. In 1849 Magnus Huss noted that the blood vessels of an alcoholic are subject, "to the so-called atheromatous process, whereby there is peculiar deposition beneath their innermost membrane, giving rise to the development of a variety of other changes, such as ulceration, aneurysms, rupture and angioliths. At the end of the 19th Century alcohol was regarded as one of the causes of atherosclerosis. Cabot presented data in 1904 indicating that atherosclerosis was actually rare in alcoholics dying before the age of 50 This conception soon predominated and was furthermore supported by a number of studies conducted in the middle of this century On the basis of material comprising 782 cases of portal cirrhosis, Hall *et al* (1953) was able to report that atherosclerosis in the aorta of males was slightly less than and in the coronary arteries, only half as frequent as in a control group. Grant *et al* (1959) found that only 3.3 % of the subjects with portal cirrhosis verified at autopsy had died of acute coronary occlusion with myocardial infarction as compared to 11 4 % in the general autopsy population. Howell and Manion (1960) reported that recent or old myocardial

Risk factors defined here as characteristics, traits and abnormalities which are statistically associated with an increased incidence of subsequent IHD

infarction was less than one fourth as common in autopsies of subjects with portal cirrhosis as in non-cirrhotic patients. Similarly Ruebner *et al* (1961) found myocardial infarction in 3 % of 399 subjects with portal cirrhosis at necropsy as opposed to 11 % in the same number of controls matched for age, sex and race

Medical opinion on portal cirrhosis and IHD current in the early sixties was summarized by Stare (1961) who concluded "Whatever the mechanism may be, portal cirrhosis is accompanied by a sparing of the vascular intima, particularly of the coronary circulation. However this opinion was opposed at about the same time. In reporting material from Garland, Björck (1963) suggested that minute dysfunction of the liver may perhaps promote coronary heart disease major damage, as in old alcoholics, may protect against it. In a study of 133 cases of sudden IHD death, Bainton & Peterson (1963) found that 36 subjects (27 %) were frank alcoholics or habitually consumed at least 30 oz. (85 cl) of whiskey or its equivalent per week. These results failed to disclose any prophylactic value of alcohol against IHD An electrocardiographic study by Nielsen & Kessing (1965) of 100 subjects with definite signs of portal or postnecrotic cirrhosis was unable to confirm an increased frequency of signs of IHD compared with a matched control group Hirst *et al* (1965) objected to the extension of conclusions based on findings in cirrhotic patients to alcoholics in general. They compared the frequency of atherosclerosis and myocardial infarction in cases of chronic alcoholism with and without portal cirrhosis and found that alcohol consumption gives no protection against atherosclerosis but that cirrhosis may do so

The number of investigations pointing to a link between alcohol consumption and IHD has increased since 1965 Davies (1965) reported that death from atherosclerotic and degenerative heart disease is 2.63 more frequent in alcoholics than in controls. The mortality ratio for Sundby's (1967) comprehensive study showed that death from IHD was 1 73 more frequent in 1 722 treated alcoholics than in the general Norwegian population. The non-vagrant group showed consistently higher mortality rates than the "vagrant group throughout all the years of the observation. Sundby concluded that alcohol does not "have any beneficial effect upon the degenerative vascular progress. The evidence rather points to an opposite effect, whatever the explanation. Significantly increased IHD mortality ratios in alcoholics have also been reported by Schmidt & de Lint (1972) and Pell & D Alonzo (1973) "Known, suspected and recovered alcoholics in the latter investigation were carefully matched with controls with respect to age, sex, income group and geographical location. This sample was repeatedly followed for five years, and it could also be shown that hypertension was found 2.3 times more often in the alcoholics than in the controls.

Subjects surviving myocardial infarction also appeared to consume more alcohol than average. In a subject group comprising 72 males surviving myocardial infarction Villiger & Hayden (1966) found that 74 % of the subjects satisfied the criteria for major or minor alcohol abuse as opposed to 28 % of the control group. In a Swedish study of men born in 1913 Tibblin (1972) found that the incidence of IHD over a 9 year period in the group with alcohol problems —defined as registration at the local Temperance Board* —was 3 times greater than in the complementary group. The mortality rate for registered smokers was more than 6 times higher than for non-registered non smokers. Analysing Tibblin's material (1967 1972) by means of a multiple logistic model, Wilhelmsen *et al* (1973) found that registration at the Temperance Board, which represents a rather rough indication of drinking habits, was an independent risk factor for acute nonfatal myocardial infarction and acute fatal IHD Friberg *et al* (1973) reported that 10 % of non smokers were registered in a nationwide alcohol registry as opposed to 30 % of those smoking more than 10 cigarettes a day The data suggest that IHD mortality among registered twins,

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Sw Nykterhetsnämnden

Sw Kriminalvårdsregistret

irrespective of their smoking habits, was higher than among the non registered twins.

☆

The importance of heredity in the pathogenesis of IHD is much debated, but family and twin studies, have shown it to play an important role (Epstein 1964, 1965 Lundman 1973) Thomas (1958 1959) studying the parents and grand parents of 724 medical students, found that car diovascular disease was 2.7 times more common when both parents were affected as compared with offsprings of non-affected parents. Russek & Zohman (1958) obtained a history of definite cardiovascular disease in one or both parents of 67 % of coronary patients as compared to 40 % of the controls. Among the parents of males who had suffered a proven myocardial infarction, Rose (1964) found the IHD mortality rate to be 3 times higher than in matched controls. In a prospective study of a large number of subjects, Hammond *et al* (1971) found that IHD mortality rates were considerably higher among subjects with short-lived parents than among subjects with long lived parents.

However it must be remembered that familial aggregation of IHD is no proof of any genetic mechanism. Twin studies are better suited the task of establishing the existence of genetic factors in IHD Monozygotic (MZ) twins have genetic factors completely in common, while same-sexed dizygotic (DZ) twins have them to approximately 50 % Furthermore, twins tend to share environmental factors to greater degree than other groups (Cederlöf 1966 Lundman 1973) Verschuer (1958) found concordance rate of 19 % for coronary sclerosis among 21 pair of MZ twins as compared t 8 5 % in 47 pair of DZ twins. Harvald & Hauge (1965) analysed data from 6,893 pair of twins in Denmark. Concordance with respect to coronary occlusion was found 20 % of the MZ twins as compared to 15.5 % of the same sexed DZ. However, the difference was not significant. In a study of 92 MZ pair despite discordant smoking habits in the twin pairs, Lundman (1966) was able to show a coincidence rate of 18 5 % with respect to clinically evaluated or exercise ECG-diagnosed IHD The anticipated coincidence was 9 6 % A significantly higher concordance rate for angina pectoris was found among MZ than among DZ twins (Cederlöf *et al* 1967) in the Swedish Twin Registry (Cederlöf 1966) Liljefors (1970) also found a higher con cordance rate for MZ than for DZ twins when ST-segment depression during exercise was in cluded as a criterion of IHD

☆

Alcoholism has long since been observed to run in families. Both Amark (1951) and Winocur *et al* (1970) studying the alcoholic incidence in first degree family members of alcoholics, found alcoholism rates exceeding those anticipated for the general population. Schuckit *et al* (1971) per formed an investigation utilizing half-siblings of alcoholic parents. The results indicated that the presence of a biological, alcoholic parent predicted alcoholism in the half-sibling sample, while living with an alcoholic parent figure did not.

Twin studies dealing with alcoholism and alcohol consumption have been performed by Kaij (1960) who published a report on 174 male twin pairs in Sweden. At least one twin in each pair was an alcoholic registered at the Temperance Board. The concordance rate for alcohol consumption in the whole twin sample for MZ versus DZ twins was reported at 54 and 28 % respectively As the degree of alcohol abuse increased, the concordance rate was found to increase among the MZ but not among DZ twins. Partanen *et al* (1966) used a cohort of Finnish twins for a study of alcohol consumption and alcohol habits in 902 male twin pair. They concluded that there is a heritable influence determining the frequency and the amount of drinking in the sample and a "lack of control for the younger group, but there were no differences between MZ and DZ twins with regard to social complications. From answers to a questionnaire mailed to 1,500 same-sexed twins Jonsson & Nilsson (1968) found that both a

large consumption and abstinence were influenced to some extent by genetic factors. Thus, the three studies reported are consistent with a genetic influence on drinking patterns.

☆

Great interest has been devoted to the relationship between *excessive* alcohol consumption and specific somatic responses. Recent studies, using an epidemiological approach involving large numbers, noted that subjects with often extreme alcohol consumption were more prone to develop IHD. The purpose of the present study with its clinical approach was to examine the extent to which moderate, longstanding alcohol consumption is accompanied by somatic deterioration and if it gives rise to factors associated with IHD. Overt IHD has a relatively low prevalence in middle-aged Swedish men (e.g. Tibblin 1967, Biörck 1968, Stamler 1973). Consequently its investigation in such a population would require a very large number, such as the Twin Registry can not yet provide. Hence, the prevalence of precursors of IHD has been investigated. As genetic factors have an influence on the development of IHD, it has been considered important to minimize the influence of the hereditary background as much as possible. Therefore a subject group comprising twin pairs was selected, i.e. twin pairs differing with respect to alcohol consumption. Few alcohol-discordant monozygotic pairs have been traced, since monozygotic twins have shown high concordance in alcohol consumption. However, since about 50 % of genetic factors are shared in common, same-sexed dizygotic pairs are also of considerable value. In addition to this advantage, the twin approach also means that the influence of early environmental factors is reduced. Thus, a twin comparison must be considered superior even to comparisons based on matched pairs. The hypotheses set up for the present study were the following:

1. Moderate longstanding alcohol consumption is accompanied by a gain in weight, an increase in subcutaneous fat thickness, high tobacco consumption, elevated blood pressure and elevated values for serum lipids, blood glucose (with a pathological intravenouse glucose tolerance test) and serum uric acid.

2. There is a connection between longstanding, moderate alcohol consumption and IHD diagnosed according to the case history and electrocardiographic examination.

Twin methods and selection of subject group

The special properties of twin populations in medical research provide exceptional opportunities for control of hereditary and environmental variables. Monozygotic (MZ) and dizygotic (DZ) twins would probably even share a common environment to a higher degree than other subjects. Control opportunities of this kind can not be achieved with groups selected at random.

THE CLASSICAL TWIN METHOD

The classical twin method is based on the assumption that members of any MZ twin-pair share their common environment to the same extent as members of any DZ twin-pair. If this is the case any resemblance between MZ twins which is greater than the resemblance between DZ twins must be ascribable to genetic factors. Furthermore any differences between the members of a MZ pair are considered to be the result of dissimilarities in their environment. The rationale of this approach has been discussed by e.g. Östlyngen (1949) Gedda (1961) Harvald & Hauge (1965) and Cederlöf (1966).

The classical twin method has not escaped criticism. It has never been proved that environmental intra-pair differences are the same for MZ and DZ twins.

THE TWIN CONTROL METHOD

In 1942 Gesell introduced an experimental method which he called "the twin control method. The method involved the exposure of one of the MZ twins to certain treatment, the unexposed co-twin serving as a control. This was a clearcut experimental method using "matched pairs" and manipulating stimulus factors. Since MZ twins have the same genetic origin, there were no constitutional differences between subjects and controls. Glass (1954) has used the method on a small scale in pharmacological experiments. Dencker (1958) studied the sequelae of head injuries affecting one MZ twin and used the un-affected co-twin as a control. The co-twin control method has been used relatively seldom, despite its many advantages.

A different situation was found in studies of smokers and nonsmokers conducted by Cederlöf *et al.* (1966) and Lundman (1966) who compared the frequency of certain chronic disorders in exposed and non-exposed partners. Smoking can not be regarded as a randomly induced habit but results from "self-selection. Kaij (1960) also used the co-twin control method to study the occurrence and structure of chronic alcoholic deterioration by comparing MZ and DZ twins with discordant drinking habits. Liljefors (1970) studied the frequency of factors associated with IHD in IHD-discordant twin pairs. Thus, application of the co-twin control method in this manner is only partly analogous to an experimental model.

To sum up twin studies possess two general advantages firstly the classical co-twin method provides the investigator with information as to whether a disease has a genetic influence, and secondly if this is the case makes it possible to keep hereditary factors constant when the effect of specific environmental exposure is evaluated.

THE SWEDISH TWIN REGISTRY

The present group of subjects was assembled using the Swedish Twin Registry compiled in 1961—1962 at the Department of Environmental Hygiene, Karolinska Institute and the National Institute of Public Health. The registry comprises about 10,000 sets of same sexed twins (about 4,500 male and 5 500 female pairs) and covers about 95 % of all Swedish same-sexed twins born

TABLE 1 Distribution of examined twin pairs by age and zygosity

	AGE					
	45—50 yrs	51—55 yrs	56—60 yrs	61—65 yrs	M(SD)	Total
MZ	2	4	4	4	56.9(5.2)	14
DZ	15	19	17	5	54.6(4 6)	56
MZ+DZ	17	23	21	9	54.6(4 8)	70

in the country from 1886—1925 and still alive at the time of complication. About 35 % of the twin pairs were judged to be MZ. The registry has been presented in detail by Cederlöf (1966) and Cederlöf *et al* (1970) Data were mainly collected using three different questionnaires mortality is registered continuously (Friberg *et al* 1970 Friberg *et al* 1973) Two clinical studies of sub-samples have also been made (Lundman 1966, Liljefors 1970)

Information was obtained from the question naires, mailed to the entire twin population, on zygosity smoking, angina pectoris, respiratory symptoms and a number of socio-economic vari ables. Information on alcohol consumption was also included. The present group of subjects was selected on the basis of this information. The questions pertaining to alcohol consumption originated from a questionnaire originally distri buted in 1967 The mailed questionnaire, returned by the twins in 1967 consisted of questions in volving the frequency and amounts of certain types of alcoholic beverages consumed Thus, consump- tion was based on a frequency-quantity index, ex pressed in grams of absolute alcohol (Appendix, page 92)

CRITERIA FOR SELECTION AND THE SUBJECT GROUP

The material was classified according to the following drinking discordances, based on the answers of the 1967 questionnaire > 10 000 < 2,000 5 000—10 000 0—400 and 2,000—5,000 grams of absolute alcohol/year no con- sumption whatever About 70 % of the target male population answered the 1967 questionnaire. In total 86 male twins pairs, approximately 25 % of whom MZ and 75 % DZ twins, of slightly more than 1 700 pairs, fulfilled the first criterion. Another 83 twin pairs fulfilled the other two criteria. All of the 86 highly discordant twin pairs were invited to take part in a clinical examination at the Serafimer Hospital. Another 6 twin pairs living in the Stockholm area who fulfilled the second criterion above and showed a high level of alcohol discordance, were also included in the examination. According to these criteria the mem- bers of each pair were assigned to a high alcohol group (HAG) or low alcohol group (LAG) A to- tal of 92 pairs, from 45—65 years, were invited to participate in the study and 70 (76 %) complete pairs were examined. Fourteen of these pairs were MZ and 56 DZ twins. The distribution of the series with respect to age and zygosity is shown in Table 1

Comments

Even though the Swedish Twin Registry pro- vided a large material for selection, only 86 pa tently alcohol-discordant twin pairs could be found. That the number is small is mainly due to a high rate of concordance among twins in the answers given concerning alcohol. Alcohol-discordant pairs were especially uncommon among MZ twins. These findings were consistent with the genetic influence on drinking patterns demon- strated in earlier twin studies (Kaij 1960 Parta nen *et al* 1966, Jonsson & Nilsson 1968) Another factor which may have affected the selection is the presumed rather high drop-out rate for questions pertaining to alcohol. It is reasonable to assume

that some highly alcohol-discordant pairs were lost on account of this reason.

Only male twin pairs were selected for the study because the number of females with alcohol discordant drinking habits in the Twin Registry was regarded as small, and because more serious forms of IHD such as myocardial infarction and sudden IHD death, are less frequent among women than men (e.g. Epstein 1965 Kannel *et al* 1967b, Simborg 1970 Kuller & Tonascia 1971 Stamler 1973 Bengtsson 1973)

Since examined twins in ideal circumstances should have the same background according to the area of residence, this background was analysed in the subject group differences were found in 6 pairs. Four of the high consumers resided in urban areas while the low consumers resided in rural areas. The opposite was found in 2 pairs. Thus, no dissimilarities with respect to area of residence could be shown between the members of the twin pairs This eliminates the environmental influence caused by area of residence said to affect the cardiovascular system (Biörck 1959 1963 Epstein 1965 Simborg 1970 Stamler 1973)

TIME AND PLACE OF INVESTIGATION

The investigation was begun in February 1972 and completed in March 1973 Most members of twin pair were examined in different weeks but, in principle, on the same day of the week. All the twins were examined at the Serafimer Hospital, Stockholm.

Each twin arrived at the hospital at 7.45 a.m. and had completed the medical screening by 12 p.m. After lunch, the twins were interviewed by a psychologist and psychiatrist. Interviews took a total of 1—2 h for each subject. Thus, the twins were able to leave hospital between 2 and 3 p.m. Only one twin was examined each day

Different population studies have shown that subjects with a particular disease are more inclined to submit to medical examinations than others (Cobb *et al* 1957) To avoid this bias, the twins were not informed that this study was concerned with IHD or about the specific effects of alcohol on the cardiovascular system.

NON-RESPONSE

Twenty-two pairs (24 %) were not available for study (Fig 1)

One or both of the twins in 8 pairs had died in 1967—1972. All of the deceased had been subjected to autopsy examination. The causes of death, according to the pathologist's report, are presented in Table 2.

Five subjects died both in the high alcohol group (HAG) and in the low alcohol group (LAG) Four of the subjects in the HAG and the same number in the LAG group died before their co-twins. Diagnoses associated with IHD were found in 3 of the twins in the high alcohol group and in one of the twins in the low alcohol group

Five pair did not participate in the investigation because of severe illness. The diagnoses reported were cerebral infarction with hemiplegia, poliomyelitis, grave epilepsy and debility in two cases. Low alcohol consumption was present in 4 out of 5 pair among these sick patients.

TABLE 2. Causes of death leading to non-response by alcohol-discordant twins

	High Alcohol Group (HAG)	Low Alcohol Group (LAG)
1	Fibrosis myocardii + Atherosc. coronariae	Uremia + Hypertonia + Sepsis (myocardium and coronary arteries N.A.D.)
2	Tumor pulm c. metastatibus multiple	Abs
3	Infarctus cordis + Cardiosclerosis	Abs
4	Abs	Insuff cordis + Hyperthyreosis
5	Strangulatio + Depressio mentis	Abs
6	Abs	Myelomatosis
7	Abs	Bronchopneumonia + Fractura cranii
8	Insuff cordis acutus + Morbus cordis arteriosclerotisus	Cancer ventriculi c. metastatibus

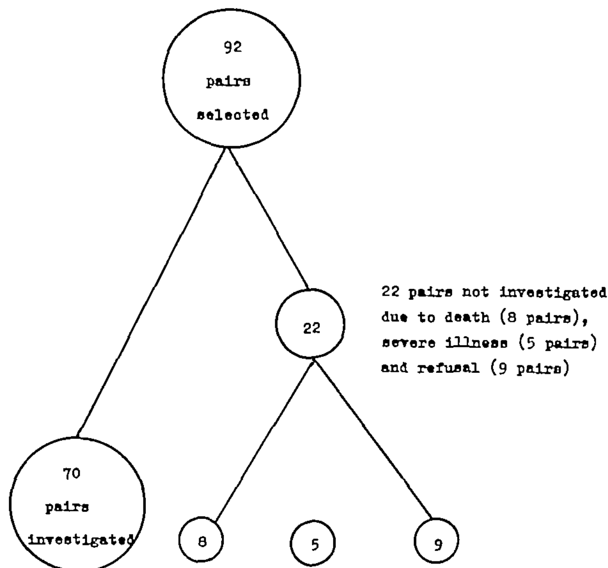


Fig. 1. The Subject Group

Only 9 of the 92 (9,8 %) twin pairs refused to take part. In most cases this was because their work presented an obstacle, but unwillingness, fear or the like also played a part.

Comment

The number of non participants must be regarded as low especially when considering that many of the subjects had to travel more than 300 miles to reach the examiner. The non respondents were fairly evenly distributed with respect to age and zygosity as compared to the subject group. It is not possible to be certain how non respondents biased the final results. All of the living non-participants were contacted by telephone. In many cases it was obvious that these pairs included a member who was unable to make the journey to the investigator because of alcohol consumption. Therefore, it may well be that the possibility of disclosing differences between the various exposure groups has been reduced.

ZYGOSITY DIAGNOSIS

Zygosity in the Twin Registry has been estimated on the basis of replies to questions relating to resemblance, viz. alike as two peas in pod as children. If both twins agreed that they had been as alike as two peas in pod, the pair was considered to be MZ. If on the other hand, they only admitted to a family resemblance the pair was regarded as DZ. The validity of this method has been examined by Cederlöf *et al* (1961) Zygosity was verified in 95 % of the MZ and 90 % of the DZ twins by means of serological methods five different grouping systems were used.

Serological determinations were performed on all twins in the present study It was carried out at the National Laboratory of Forensic Medicine and comprised the blood group systems ABO Rh, MN P K, Le, Fy Jk and the serum groups Hp, Gc, Ag, Gm, Inv PGM, EAP BA AK, ADA, and PGDA. When there were differences in zygosity grading between the Twin Registry and the serologic determination, the laboratory investigation was considered to be the most valid. All of the 54 initially DZ pairs had the same zygosity according to serological determinations, and 14 of the 16 initially MZ pairs showed the same serological zygosity Two twin pairs had been classified as MZ in the Twin Registry but as repeated serological tests displayed different serum groups, they were classified as DZ in the present study

Comments

The smaller the subject group, the more important the determination of zygosity Correct identification of zygosity can only be obtained in pairs in whom a common chorion membrane is found. Serological determination in the present study must be regarded as the most valid method for zygosity determination. The number of blood group systems and serum groups used in the present study group was large increasing the reliability of adequate zygosity determination. The resemblance between the results obtained by the Department of Hygiene and the National Laboratory of Forensic Medicine strengthened the zygosity diagnosis.

The number of twin pairs with respect to zygosity thus showed 14 MZ and 56 DZ pairs. It is clearly not possibl to evaluate the nfluence of genetic factors with this small material. Nevertheless, the material classified by zygosity groups will be described. The results will be presented in terms of the pooled zygosity group (MZ+DZ) However it was considered of some interest to see whether or not the small number of subjects in the MZ group would follow the same tendency as found in the DZ group.

General methods and procedure

Case history

In order to avoid investigator bias, each subject's case history was taken after the physical examination and without information of the results of laboratory tests. Nor did the investigator know the individual answers to the mailed questionnaire sent out by the National Institute of Public Health 1967. He was thus completely ignorant of the drinking habits of the examined subjects. The interview consisted of a sociological and a medical part. About 250 different items were discussed. Thus, the attention of the subjects was not focused on questions concerning alcohol.

Sociological interview

The sociological interview was conducted by the author. Standardized questions pertaining to family conditions, education, occupation, work, smoking, diet and physical exercise were included. The amount of alcohol consumpted and the pattern of "alcohol behavior" were thoroughly assessed. The questions were more detailed than in the 1967 questionnaire (Appendix, p. 92) to obtain an accurate measurement of present alcohol consumption. The methods used in the evaluation of the alcohol questionnaire are presented in detail in chapter III. Since a separate report on the subject group's sociological, psychological and psychiatric variables is to be published by the Department of Psychiatry, S:t Göran's Hospital, and the Department of Medicine, Serafimer Hospital, Stockholm, no information on these items will be provided in this account. It can be mentioned that no disparities were found between high and low alcohol consumers as to e.g. income and how many of them were gainfully employed. No less than 90 % of the investigated subjects in both groups were working.

Medical interview

Before his arrival in hospital the subject had already received a questionnaire about his previous and present state of health. This consisted of a general and a cardiovascular section. The answers were analyzed by the author in an interview to ensure that all questions had been properly answered.

Questions concerning *respiratory symptoms* were in part the same as those drawn up by the British Medical Research Council's Committee on the Aetiology of Chronic Bronchitis (1960).

Cough was diagnosed when morning cough was present regularly.

Phlegm was diagnosed when morning phlegm was present regularly.

Asthma was diagnosed when it had been diagnosed by a physician or when typical evidence of asthma was found in the medical history.

Dyspnea was classified in 5 grades, but the results for grades 1—2 and 3—5 are presented together. Grade 2 denoted breathlessness when hurrying on level ground or walking up a slight hill and grade 3 shortness of breath when walking with other people of same age on level ground (Rose & Blackburn 1968).

The *cardiovascular* questionnaire was in part identical with the questionnaire designed and tested for several years at the London School of Hygiene and Tropical Medicine (Rose 1962).

Myocardial infarction was diagnosed after Hofvendahl (1971):

1. Central chest pain for $\geq$ 30 min combined with at least one of the following criteria.

a) The appearance of a pathological Q wave and/or the appearance or disappearance of a localized elevation of ST segment, followed by inversion of T waves in at least two ECG leads.

b) Laboratory indications of a myocardial dam

age two SGOT values of 40 U or more achieving peak value about 24 h after the onset of symptoms, in combination with lower SGPT values, reaching a peak after about 36 h, and/or two HBD (alpha-hydroxybutyrate dehydrogenase) values exceeding 75 % of corresponding LDH (lactic dehydrogenase) values exceeding 400 U with a peak about 60 h after the onset of symptoms or combination of one SGOT SGPT combination and one HBD-LDH combination, elevated as stated above.

2. Diagnosis at autopsy

Suspected infarction existed if the subject had suffered central chest pain lasting for at least 30 min unaccompanied by the above signs of myocardial infarction (Rose 1962)

Angina pectoris was defined as a pain in the sternal region, with or without radiation into the arms or towards the jaw or pain to the left or anterior chest radiating into the left arm, accompanying physical exertion and disappearing or subsiding within 10 min after resting or taking nitroglycerine medication (Rose 1962)

Chest discomfort existed when some but not all of the aforementioned criteria for angina pectoris were fulfilled.

Palpitations bradycardia and irregular heart rate were considered to exist when reported by the subject.

Intermittent claudication was diagnosed according to Rose (1962)

Suspected intermittent claudication was diagnosed when some but not all of the stated criteria were fulfilled and when intermittent claudication was strongly suspected

Comments

The use of identical questions concerning different symptoms and other items will increase the validity of intra pair comparison, as compared to an unstructured interview (Rose & Blackburn 1968) The diagnoses of angina pectoris and intermittent claudication were based on criteria recommended by Rose (1962) and the WHO Expert Committee on Arterial Hypertension and Ischaemic Heart Disease (Burgess et al 1963) In a validity test, Rose (1961) found that uniform questionnaires greatly reduced intra- and inter-observer variability in the diagnosis of angina pectoris.

Physical examination

The following variables were examined in addition to an examination of general appearance lymph nodes, the mouth and throat, heart, peripheral arterial pulses, lungs, abdomen, thyroid gland and tendon reflexes.

Anthropometric variables

Weight was recorded to the nearest kilogram and included light underclothes.

Overweight was determined according to a modification of a weight/height index described by e.g. Frisk et al. (1959)

$$\frac{\text{weight (kg)}}{0.9\ (\text{height, cm-100})}$$

Skinfold thickness was determined by measurements in the subscapular and triceps areas on the right side of the body A Harpenden skinfold caliper (Keys 1966) was used. The pressure applied by the caliper was 10 g/mm² and the area of the contact surface was 20 mm² The skin and subcutaneous tissue were gripped with finger and thumb 1 cm above the point of measurement and squeezed firmly The skin fold was measured to the nearest 0.1 mm.

Blood pressure measurements

The blood pressure of all subjects was measured with the same mercury manometer between 9—10 a.m. The procedure was performed in the same room, and noise and chilling were avoided. The pressure was measured in the right upper arm with the subject in a supine position. An adhesive cloth sleeve measuring 35×13 cm was used. Korotkoff's auscultatory method was used, the systolic pressure being read at the appearence of the sound and the diastolic at disappearance. At least two measurements were performed. If the values were not identical the procedure was repeated, the lowest value then being recorded. The reading was made to the nearest 5 mm Hg.

Blood pressure was recorded at the start of the

examination (casual blood pressure) and after 15 min of rest in complete quiet (basal blood pressure)

Comments

Sources of error in sphygmomanometry can be roughly divided into three categories firstly the design of the cuff and variations obtained with respect to arm circumference and cuff width, secondly the fact that different examiners taking the blood pressure of the same subject may obtain different readings thirdly possible differences in the interpretation of Korotkoff's sounds (Rose & Blackburn 1968 King 1969)

The results of three different studies on cuff dimensions (Karvonen *et al* 1964 Simpson *et al* 1965 King 1969) were in close agreement, indicating that long-bladdered cuffs produced the smallest range of random errors irrespective of arm-circumference. All these studies recommended a cuff containing a bladder at least 40 cm long. The most important factor in indirect blood pressure measurement is intra and inter-observer variability The latter was eliminated in the present study since all the determinations were made by the same person (the author)

Diastolic pressure in the subject group was measured when Korotkoff's sounds disappeared (Phase 5) Karvonen *et al* (1964) and King (1969) found that this measurement agreed the closest with the values obtained by intra arterial means.

Some somatic definitions

Dupuytren's contracture was defined as a contracture of the palmar fascia.

Erythema of the palms was defined as an exaggeration of the ordinary speckled mottling of the palms.

Gynecomastia was considered present when a fleshy firm disc of tissue with well-delineated borders was palpable in the substance of the breast.

Hepatomegaly was defined as a liver palpable at least 3 cm below the right arcus in the mid clavicular line.

Hypertension was defined as a history of high blood pressure treated with anti-hypertensive agents.

Tremor was defined as course finger tremble with a larger-than normal amplitude found in writing or when the subject held a piece of paper between two fingers.

Vascular spiders— Paper money" skin was defined as more than three spiders or numerous small vessels present in the vascular territory of the superior vena cava.

Jaundice was defined as true or suspected yellow sclerae

Laboratory tests

Blood and urine tests

Blood samples were taken from the subjects in the morning following an overnight fast. A urine specimen was collected the same morning. All analyses, with the exception of blood alcohol, were performed at the Department of Clinical Chemistry Serafimer Hospital. Serum was obtained by centrifugation. E.S.R., serum creatinine hemoglobin, serum aspartate aminotransferase (SGOT) serum alanine aminotransferase (SGPT) serum alkaline phosphatase (SALP) white blood cell count, platelets, serum lipid electrophoresis, blood alcohol and blood glucose were determined. Twenty ml of serum was stored in a freezer for subsequent concomitant analysis of the members of the twin pairs with respect to serum lipids and uric acid. Twin pairs discordant as to > 1 000 grams of absolute alcohol/month were also subsequently and at the same time analysed with regard to serum gammaglutamyl transpeptidase (SGT) Investigations for glucose, protein and sediment were made on urine samples.

SGOT and SGPT were analysed using a Reaction Rate Aanalyser (LKB 8600) connected to an evaluation unit (Optilab Bo Philip Instrumentation, Stockholm) Reagents for SGOT and SGPT were obtained from Kabi AB Stockholm. Reference values. SGOT and SGPT ≤ 35 U/l

SALP was analysed according to Bergström & Thunblad (1970) Reference value SALP ≤ 35 U/l

SGT was analysed according to Szasz (1969). Reagents were from Roche Diagnostica, Basel. Reference values ≤ 40 U/l

Blood glucose was determined in capillary blood samples from the finger tip using a glucose oxidase method. Reagents were GLOX NOVUM obtained from Kabi AB, Stockholm, 1972. Reference values 55—100 mg/100 ml.

Chol sterol was determined with an Autoanalyzer according to the method of Levine & Zak (1964). Reference values 140—290 mg/100 ml.

Triglycerides were determined with an Autoanalyzer according to the method of Kessler & Lederer (1965). Reference value < 180 mg/100 ml.

Lipoprot in electrophoresis in agarose gel was carried out in accordance with a method described by Noble (1968). Normal range: Alfa lipoprotein 25—50 % beta lipoprotein 40—60 % pre-beta lipoprotein 5—25 %.

Uric acid was determined with an Autoanalyzer according to the method of Hawk *et al.* (1964). Reference value in males < 7.5 mg/100 ml.

Blood alcohol was determined, according to Goldberg & Rydberg (1965) at the Department of Alcohol Research, Karolinska Institute, Stockholm.

Glucose tolerance test

The intravenous glucose tolerance test (IVGT) was used, as described by Wahlberg (1966). Participants were asked not to eat nor take any medication after 8.00 p.m. the day preceding the test. All tests were started at 8.00 a.m., and subjects rested for at least 15 minutes before the start of the test.

After taking duplicate capillary blood samples for glucose determination, 25 grams of glucose in a 50 % aqueous solution were injected intravenously over a period of 2 to 4 min. Zero time was set at the end of the injection. Blood samples were then taken every 10th minute for 60 minutes, the samples at 20, 40 and 60 minutes duplicated. The subjects were kept supine throughout the test. The half life of blood glucose in minutes ($t_{\frac{1}{2}}$) was determined with an electronic desk calculator (Hewlett Packard) which also calculated the k value according to the equation previously described by Ikkos & Luft (1957), Wahlberg (1966) and Paasikivi (1970).

$$\text{k value} = \frac{0.693 \times 100}{t_{\frac{1}{2}}}$$

In accordance with the two latter authors, the following IVGT classification was used:

Diabetic IVGT k value	≤ 0.90
Borderline	0.91—1.10
Normal	≥ 1.11

Comments

The IVGT was chosen instead of the oral glucose tolerance test (OGTT) for the following reasons. The IVGT had been used at Serafimer Hospital since 1960 and found to be reproducible in patients over intervals of days and years (Paasikivi 1970). Furthermore, the test is simple to perform and less time-consuming than the OGTT. Also the results are easy to express and convenient for statistical analysis. The use of a desk calculator for estimation of k values probably avoided methodological errors in the original graphical technique.

Chest X-rays

A chest X-ray with the patient standing was taken according to Liljestrand *et al.* (1939). The relative heart volume, expressed in ml/m² of body surface area (BSA), was calculated (Jonsell 1939) by an experienced radiologist (Leif Frman, M.D.) who also examined the heart configuration and lung films. The upper limit for heart size in men is 500 ml/m² of BSA, at the X-ray Department of Serafimer Hospital.

Electrocardiographic examination

The clinical examination included ECG records at rest and, in all cases but one, during exercise. The recordings after exercise were made immediately, 3 and 10 minutes after the test. The instrument was a direct recording 6-channel electrocardiograph (Mingograf 61, Elema Schönander AB, Stockholm). The ECG before and after work was recorded with the subject in supine position, and

the following leads were used I—III, aVR, aVL, aVF and 6 chest leads (CR_4R, $CR_{1,2,4,5,7}$) The chart speed was 50 mm/s.

The exercise ECG was recorded with the subject exercising on an electrically braked bicycle ergometer (Elema-Schönander AB Stockholm) The initial load was always 50 Watts. The load was increased stepwise at 6-min intervals until the subject was exhausted, developed angina pectoris or showed ECG changes (arrhythmias, STsegment changes etc.) To avoid interference from muscle action potentials during the test, the reference electrode was placed on the forehead according to Holmgren & Strandell (1961) During exercise the ECG was recorded using a chart speed of 10—25 mm/s. The heart rate was calculated from the ECG

Interpretation of ECG

The ECG findings were coded according to a modification (Åstrand *et al* 1967) of the Minnesota code (Blackburn *et al* 1960)

The ECG was interpreted at the highest comparable heart rate so as to facilitate intra pair comparison of the ECG response during exercise This was defined as the highest heart rate recorded by the twin with the lower final heart rate in a pair. All ECG examinations were interpreted by the author over a continuous period of about 2 weeks and without any knowledge of the subjects identity Several ECG recordings were coded twice to ensure that the criteria had not changed during the period of interpretation. Torbjörn Lundman, M.D kindly interpreted several of the ECG tracings to confirm that the interpretation agreed with that of an experienced cardiologist. In accordance with Blomqvist (1965 1971) and Åstrand (1973) segmental ST depressions according to ST code 1—3 (Table 3) were considered indicative of subclinical IHD

Comment

Inter-observer variations are greater than intra observer variation in ECG interpretation (Åstrand 1965) She found close agreement for ST depressions according to ST code 1—3 and slightly poorer results according to ST code 4, in repeated coding of 200 ECG records.

TABLE 3 Electrocardiographic code for ST depressions

Code number	ST junction and segment measured in leads I, II, VL, VF CR_{1-7}
0	Normal ST segment
1	ST J depression of at least 1.5 mm or at least 1.0 mm and ST segment straight and slowly ascending, horizontal or downward sloping
2	ST J depression of 1.0—1 4 mm and ST segment straight and slowly ascending, horizontal or downward sloping
3	ST J depression of 0.5—0 9 mm and ST segment straight and slowly ascending, horizontal or downward sloping
4	ST J depression less than 0.5 mm but ST segment downward sloping and reaching 0.5 mm or more below P R baseline
5	ST J depression less than 0.5 but ST segment horizontal or downward sloping and reaching less than 0.5 mm below P R baseline
6	Isolated ST J depression of at least 1.5 mm or at least 1 0 mm ST segment upward sloping
7	Isolated ST J depression of at least 0 5—1 4 mm or at least 0 5—0 9 mm ST segment upward sloping

Statistical methods

Conventional statistical methods have been used for the calculation of the arithmetic mean (M) and standard deviation (SD)

With respect to intra pair comparison of qualitative variables, such as symptoms, diagnoses and ECG-findings, concordant and discordant pairs were expressed in absolute numbers, as the example given below

		Low alcohol group	
		Affected	Un-affected
High alcohol group	Affected	a	b
	Un-affected	c	d

The statistical analysis refers to the cells b and c which consist of the numbers of twins discordant with respect both to alcohol consumption and to the dependant variable. Cell b expresses the number of twins in the high alcohol group positive to a trait when a co-twin is not. Cell c on the other hand, expresses the number of twins in the low alcohol group positive to a trait when a co-twin is not. The assessment of statistical significance deals with the relationship between these two figures. The one tailed hypothesis that the twins in the high alcohol group had a greater prevelence of positive findings was tested using McNemar's test (Siegel 1956). The Binomial test was used in cases with less than 10 discordant twin pairs (Siegel 1956).

The mean intra pair differences for quantitative variables in relation to alcohol consumption were examined using Student's paired "t"-test. The following equation was used

$$t = \frac{\bar{d}}{\sqrt{\frac{SD^2}{n}}}$$

in which $\bar{d}$ is the mean of intra-pair difference, n the number of pairs observed and SD_d the standard deviation of the differences between the pairs of observations. Degrees of freedom are n—1

It is not self-evident that quantitative variables are statistically best evaluated by using an intra pair difference method. It might well be that a small number of pathologically altered values may only affect mean group values to a minute degree while increasing variance considerably. In cases in which conventional cut-off points between normal and pathological conditions can be defined, a qualitative assessment may be statistically more efficient. Many quantitative variables were also subjected to qualitative assessment in the present study.

The null hypothesis of no difference was rejected at the significance level of 5 % ($p < 0.05$ *), 1 % ($p < 0.01$ **) and 0.1 % ($p < 0.001$ ***)

Evaluation of alcohol discordance and validity of alcohol classification

The twins in the 1972/73 investigation were carefully interviewed with respect to alcohol consumption and alcohol habits in an effort to analyse the validity of the information obtained from the 1967 questionnaire (Appendix, p. 92). Furthermore, physical findings and some determinations of serum enzymes associated with a high alcohol intake were also investigated.

Alcohol in blood was determined (p. 21) to check the fasting status. Only one subject had detectable alcohol (1.4 ‰). He belonged to the high alcohol group.

ALCOHOL EVALUATION

One bottle of beer (33 cl) was assumed to contain 12 g of absolute alcohol, one bottle of wine (75 cl) 60 g and one bottle of spirits (75 cl) 250 g (See Appendix, p. 92). Thus a subject reporting the consumption of one beer daily was assumed to have an annual consumption of 365 × 12 = 4,400 g of absolute alcohol (Wallgren & Barry III 1970, Hollstedt 1974).

Comments

The 1967 questionnaire was unsatisfactory as insufficient alternatives were provided for the frequency of alcohol consumption. Thus, just one alternative was provided— once a week —for respondents who might drink once, twice or three times a week. When selecting twin pairs, this alternative was assumed to be twice a week. This often produced too high an estimate of a subject's consumption. Furthermore, no distinction was made between the different types of beer now available in Sweden. Weak beer (class I), for example, only contains 6 g of absolute alcohol per bottle (33 cl). In the following alcohol discordance was consistently based on the last interview, i.e. the one carried out in 1972—1973. This means that the information on alcohol consumption in the 1967 questionnaire was only used to select alcohol discordant twin pairs.

REPEATABILITY

A comparison of intra pair differences expressed in grams of absolute alcohol in 1967 and 1972/73 is provided in Table 4. Forty-two of the 70 pairs investigated (60 %) in 1972/73 still displayed a discordance of more than 8 000 g of absolute alcohol/year. This difference pertains to the primary criterion for selection (> 10 000 versus < 2,000 grams/year). In 1972/73 a total of 57 pairs (81 %) displayed a difference in consumption of at least 4 000 g of absolute alcohol/year. A low discordance, i.e. less than 4 000 g, was found in 13 pairs (19 %). All pairs were classified in an identical manner in 1967 and 1972/73 with respect to the intra pair high-low relationship.

Comments

Repeatability on the basis of the intra-pair high-low relationship thus amounted to 100 %. The reduced discordance in 1972/73 as compared to 1967 may have been due to e.g. incongruities in the measurement technique. Furthermore there is almost always regression towards the mean in studies of this type (Remington & Schork 1970). There may also have been true changes in drinking habits from 1967 to 1972/73. Fairly constant drinking patterns have been reported by Jellinek (1960) and Nilsson (1973) for middle-aged men, but a decrease in consumption has been shown for older subjects (Jellinek 1960, Carlsson 1970). In any case, data point to a consistent discordance in volumes consumed in the past 6 year period.

Average alcohol consumption for Swedish adults 19 1 is said to amount to 4 500 g of absolute alcohol/year (Rapport 19 3).

TABLE 4 Intra-pair discordance expressed in grams of absolute alcohol according to the 1967 questionnaire and the 1972/73 interview

	Discordance in grams of abs alc/year	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)	
		> 8000	4000—8000	> 8000	4000—8000	> 8000	4000—8000
1972/73	> 8000	7	1	30	4	37	5
	4000—8000	2	—	12	1	14	1
	< 4000	4	—	9	—	13	—

SOME ANAMNESTIC VARIABLES

One method of determining a subject's alcohol habits is to identify certain behavior related to high consumption. Blackouts and the use of "eye openers" ("återställare") are some of the variables often used in defining alcoholism (Jellinek 1960, Carlsson 1970). These variables as well as the following were elucidated in the 1972/73 interview: drinking behaviour at parties, intoxication, hangover and "more than half a bottle of hard liquor or a bottle of wine on the same occasion", alcohol problems, violence when drunk, drunken driving, police contacts when drunk, neglected work or marriage problems due of alcohol. The last six variables are not presented because of their low prevalence. The remaining anamnestic variables are presented in Table 5.

Blackouts

A larger number of blackouts was found among the high alcohol consumers in the pooled zygosity group (MZ+DZ). Seventeen subjects in the high alcohol group (HAG) versus six in the low alcohol group (LAG) reported blackouts ($p < 0.01$). Three pairs were concordant as to this criterion.

Eyeopeners

A positive answer to the question on the use of "eyeopeners" was significantly more common among the HAG in the pooled zygosity group ($p < 0.05$). Eleven twins in this group gave a positive reply versus 3 subjects in the LAG.

Comments

Blackouts and the use of "eyeopeners" are two of the variables commonly associated with a high alcohol consumption and alcoholism (Jellinek 1960, Carlsson 1970). Blackouts may be "one of the best indicators of incipient alcoholism" (Wallgren & Barry III 1970).

Drinking behavior at parties

Four glasses of hard liquor or more at parties were considered heavy consumption. Thirty five HAG subjects versus 11 LAG subjects in the pooled zygosity group submitted positive replies on this criterion ($p < 0.001$).

More than half a bottle on the same occasion

Answers were considered positive when subjects reported drinking more than half a bottle of hard liquor or more than a bottle of wine on the same occasion at least once a month. In the pooled zygosity group 22 HAG subjects versus 4 LAG subjects gave a positive answer to this variable ($p < 0.001$).

Intoxication and hangover

In the pooled zygosity group, 20 HAG subjects versus 11 LAG subjects admitted alcohol intoxication at least once a month ($p < 0.05$). Hangover was somewhat more prevalent among the high alcohol consumers, but this finding was not significant.

Comments

During the successive development of alcohol dependence intoxication becomes an increasingly urgent desire and usually an increasingly prevalent condition (Wallgren & Barry III 1970). The same authors were less certain that a hangover is a sign of alcohol dependence. In addition, it seems

TABLE 5 Frequency f concordanc and di cordanc with re pect t om anamnestic I formation In elation t al- hol con mption

HIGH ALC		LOW ALC MZ pos	MZ neg	MZ tot	DZ pos	DZ neg	DZ tot	MZ+DZ pos	MZ+DZ neg	MZ+DZ tot
Blackouts	pos	1	2	3	2	12*	14	3	14**	17
	neg	0	11	11	3	39	42	3	50	53
	tot	1	13	14	5	51	56	6	64	70
Eyeopeners	pos	0	0	0	1	10*	11	1	10*	11
	neg	0	14	14	2	43	45	2	57	59
	tot	0	14	14	3	53	56	3	67	70
Heavy consumption at parties	pos	1	4	5	5	25***	30	6	29***	35
	neg	1	8	9	4	22	26	5	30	35
	tot	2	12	14	9	47	56	11	59	70
More than a half-bottle of alcohol	pos	0	2	2	2	18***	20	2	20***	22
	neg	0	12	12	2	34	36	2	46	48
	tot	0	14	14	4	52	56	4	66	70
Intoxication	pos	0	2	2	4	14	18	4	16*	20
	neg	0	12	12	7	31	38	7	43	50
	tot	0	14	14	11	45	56	11	59	70
Hangover	pos	0	1	1	3	9	12	3	10	13
	neg	1	12	13	7	37	44	8	49	57
	tot	1	13	14	10	46	56	11	59	70

* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

TABLE 6. Subjective classification of alcohol habits

Grade	Habit	MZ High alc.	MZ Low alc.	DZ High alc.	DZ Low alc.	MZ+DZ High alc.	MZ+DZ Low alc.
1	Total abstinence	0	2	0	3	0	5
2	Occasional drinking	1	7	3	18	4	25
3	Moderate drinking	13	5	29	33	42	38
4	Occasionally drunk often drunk-alcoholist	0	0	20	2	20	2
	Undecided	0	0	4	0	4	0
	Total	14	14	56	56	70	70

reasonable to assume that subjects less frequently exposed to intoxication and hangover are more likely to recall and report rare incidents. On the other hand subjects more commonly exposed to these conditions may have a tendency to ignore both intoxication and hangover and neglect to report on these items.

Subjective classification of alcohol habits

In the 1972/73 investigation twins were asked to describe their personal alcohol habits using one alternative on a graded scale. The results are given in Table 6. In the pooled zygosity group 30 (43 %) out of the 70 low consumers reported total abstinence or occasional drinking. The corresponding figure for high consumers was 4 (6 %). As far as moderate drinking was concerned, there was almost an equal number of subjects in the LAG and HAG i.e. 38 (54 %) and 42 (60 %) subjects respectively. The alternatives occasionally drunk — often drunk — alcoholic were choosen by 20 HAG subjects (29 %) versus 2 LAG subjects (3 %). The differences with respect to subjective classification referred to DZ twins but MZ twins also showed obvious disparities.

Comment

The extreme alternatives apparently provided discrimination of the two groups. There were 20 concordant twin pairs among subjects who chose the alternative "moderate drinking". Fourteen of these 20 pairs were found to display an alcohol discordance exceeding 4 000 g of absolute alcohol/year. It is concluded that the subjective rating certainly supports the intra pair relationship obtained from the calculation of alcohol but shows that calculated discordance expressed in grams of absolute alcohol/year is a far more efficient measure. In addition, the only subject classifying himself as being an alcoholic belonged to the high alcohol group.

PHYSICAL FINDINGS ASSOCIATED WITH HIGH ALCOHOL CONSUMPTION

The following physical findings often associated with a high alcohol intake (Sherlock 1968, Carlsson 1970, Wallgren & Barry III 1970) were analysed: hepatomegaly, tremor (coarse, confirmed), vascular spiders — paper money skin, Dupuytren's contracture, jaundice, erythema of the palms, ascites, gynecomastia and scar following stomach surgery. The last four variables are not presented due to low prevalence. The results of the examination are shown in Table 7.

Hepatomegaly

Hepatomegaly was one of the somatic findings which distinguished between the high and low consumers of alcohol. Twenty-two (31 %) of the high consumers in the pooled zygosity group were judged to have an enlarged liver as compared to 5 (7 %) of the low consumers ($p < 0.001$). Three pairs were concordant as to this criterion.

TABLE 7 Frequency of concordance and discordance with respect to some physical findings in relation to alcohol consumption

		LOW ALC									
		MZ			DZ			MZ+DZ			
		pos	neg	tot	pos	neg	tot	pos	neg	tot	
HIGH ALC	Hepatomegaly	pos	0	3	3	3	16**	19	3	19***	22
		neg	0	11	11	2	35	37	2	46	48
		tot	0	14	14	5	51	56	5	65	70
	Tremor	pos	0	2	2	1	19**	20	1	21**	22
		neg	1	11	12	5	31	36	6	42	48
		tot	1	13	14	6	50	56	7	63	70
	Spiders – paper money" skin	pos	0	2	2	1	12*	13	1	14**	15
		neg	0	12	12	3	40	43	3	52	55
		tot	0	14	14	4	52	56	4	66	70
	Jaundice	pos	0	1	1	0	5	5	0	6	6
		neg	0	13	13	1	50	51	1	63	64
		tot	0	14	14	1	55	56	1	69	70
	Dupuytren s contracture	pos	1	2	3	1	10**	11	2	12*	14
		neg	2	9	11	1	44	45	3	53	56
		tot	3	11	14	2	54	56	5	65	70

* $= p < 0.05$
** $= p < 0.01$
*** $= p < 0.001$

Comment

Fatty infiltration of the liver causing hepatomegaly as a complication of high alcohol intake, is well known (Sherlock 1968 Wallgren & Barry III 1970 Carlsson 1970) Even portal cirrhosis is often manifested as hepatomegaly (Sherlock 1968) Wiebe *et al* (1971) was able to disclose triglyceride accumulation in the liver of apparently normal subjects after a single dose of alcohol.

Tremor

Tremor was significantly more common in the HAG In the pooled zygosity group, 22 of the high consumers as opposed to 7 of the low consumers were judged to have a coarse tremor ($p < 0.01$)

Comments

Tremor is a variable phenomenon, partly under

TABLE 8 Some serum enzymes in relation to alcohol consumption.

	MZ			DZ			MZ+DZ		
	High alc. M(SD)	Low alc. M(SD)	No of pairs	High alc. M(SD)	Low alc. M(SD)	No of pairs	High alc. M(SD)	Low alc. M(SD)	No of pairs
SGOT	19.3(5.4)	18.7(4.6)	14	23.4(8.0)	19.4(4.2)	52	2.6(7.7)	19.2(4.5)**	66
SGPT	13.7(3.7)	16.3(8.0)	14	19.8(10.7)	18.0(8.9)	52	18.6(10.0)	17.6(8.7)	66
SALP	20.5(9.9)	18.1(6.6)	14	23.8(8.6)	21.7(7.6)	51	23.1(8.9)	20.8(7.5)	65

= $p < 0.05$
*** = $p < 0.001$

direct control from cerebral centers and therefore subject to the influence of mood and conscious effort. A positive relationship between finger tremor and alcohol consumption has been described by Carrie (1965) Carlsson (1970) and Wallgren & Barry III (1970)

Vascular spiders and "paper money" skin

The prevalence of vascular spiders and "paper money" skin was *significantly higher in the* HAG than in the LAG. These findings were noted in 15 of the high alcohol consumers as opposed to 4 of the low alcohol consumers ($p < 0.01$)

Comments

Arterial spiders and "paper money" skin may appear in cases of deranged hepatic function, and the appearance of fresh spiders is indicative of progressive liver damage. According to Sherlock (1968) "spiders" are especially florid in the alcoholic.

Jaundice and Dupuytren's contracture

None of the twins in the subject group displayed any signs of confirmed jaundice but suspected jaundice was generally found more frequently in *the high* alcohol *group*. No significant inter group difference was noted in respect of this finding. Dupuytren's contracture, suspected or confirmed, was more prevalent in the HAG of the pooled zygosity group ($p < 0.05$). In this group 14 of the high consumers as opposed to 5 of the low consumers were *judged to have* suspected or confirmed contracture ($p < 0.05$)

Comments

Jaundice is a sign of impaired bilirubin metabolism and may be caused by many different mechanisms, one being a high intake of alcohol (Sherlock 1968). Mild forms of jaundice are difficult to diagnose clinically and laboratory tests are necessary to confirm the diagnosis. These were not judged to be of value in the present investigation. According to Sherlock (1968) Dupuytren's contracture is sometimes a characteristic of cirrhosis in alcoholics. Other authors (Cochrane 1972, Benjafield & Rutter 1972) have stressed an autosomal dominant inheritance in this condition and that it may be linked to chronic alcoholism.

SERUM ENZYME PATTERNS

Serum aspartate aminotransferase (SGOT) serum alanine aminotransferase (SGPT) and serum alkaline phosphatase (SALP) have long been used as diagnostic aids in alcoholism and diseases of the liver (Sherlock 1968). Thus the twin pairs were analysed with respect to these aforementioned serum enzymes. The results are shown in Table 8. The twins discordant at > 1 000 g of absolute alcohol/month were also analysed with respect to serum gammaglutamyl transpeptidase (SGT). *Some of the twin pairs were not included in the* data on serum enzymes, in all cases but one owing to lost samples (3 samples for SGOT and SGPT 4 samples for SALP). This means that an whole twin was "lost" if the value of one member of a pair was lost. One pair was excluded as the low alcohol consumer long since a teetotaller had recently diagnosed liver disease following treatment

with chlorpromazine. This effect has been discussed by Sherlock (1968)

SGOT

Significant differences were found between the high and low alcohol groups with respect to SGOT Mean values n the pooled zygosity group were 22.6 and 19 2 U/l in the HAG and LAG respectively ($p < 0\ 001$)

Comments

This intra-pair comparison thus revealed a significant disparity with regard to SGOT Both groups, however, fell within normal limits. The SGOT values suggest that moderate alcohol discordance need not necessarily give rise to any serious liver damage. Bang *et al* (1958) found a rise in SGOT after acute alcohol intoxication in 27 of 35 alcoholic patients. Brohult *et al* (1966) was able to show that a single dose of alcohol, corresponding to 3 g/kg body weight, administered to apparently healthy men produced a significant rise n SGOT for a few days after alcohol intake.

SGPT

No obvious differences were obtained in the pooled zygosity group with respect to SGPT

Comments

Brohult *et al* (1966) found that the SGOT values were higher than SGPT values after a large dose of alcohol given to apparently healthy men. According to Wallgren & Barry III (1970) habitual drinkers have elevated SGOT SGPT and SALP values, while SGPT and SGT in particular are reported to be elevated in alcoholics studied during institutional treatment.

SALP SGT

Significant differences were obtained ($p < 0\ 05$) with regard to SALP Mean values in the pooled zygosity group amounted to 3 1 and 20.8 U/l in the HAG and LAG respectively

The greatest disparities between mean values were obtained for the twin pairs discordant with respect to > 1,000 g of absolute alcohol/month analysed for SGT This analysis, which has been presented elsewhere (Mythed & Bergström 1973) disclosed mean values of 33 and 11 U/l for the HAG and LAG respectively ($p < 0\ 01$)

Comments

Differences between mean values for SALP were thus not pronounced, but intra pair testing revealed significant disparities between high and low consumers. As in the case of SGOT the results support alcohol discordance but refute the presence of any serious liver damage in the alcohol positive group. Elevated SALP values have been reported in alcoholics (Wallgren & Barry III 1970) Analysis of SGT in the highly discordant mentioned group produced the most striking disparities. SGT has been termed a sensitive and specific test in subjects regarded as heavy drinkers rather than alcoholics in the determination of liver cell damage in the absence of clinical evidence of liver disease (Rosalki & Rau 1972, Rosalki 1973)

DISCUSSION

The difficulties encountered in obtaining an adequate estimate of alcohol consumption have been discussed by e.g. Kalj (1960) Carlsson (1970) and Björkman (1971) A tendency to underestimate this has been found in comparisons of subjective answers with objective measurements of volumes sold. Björkman (1971) underlined the advantage of mailed questionnaires compared to personal interviews in detecting extremes of consumption. In the present study data on alcohol consumption were not obtained in personal interviews. The respondents answered the alcohol questions by themselves. Their replies were subsequently checked in interviews. This procedure was felt to increase the valid ty of the answers.

In any study concerning the effects of alcohol on somatic status, life-long consumption would probable provide the best prerequisite for drawing any conclusions. On the other hand, fairly constant drinking behaviour has been reported by Jellinek (1960) and N'lsson (1973) in middle aged men. Information on alcohol intake in the present

study was available for two periods. The complete repeatability with respect to the intra pair high-low relationship points to longstanding alcohol discordance for the twin pairs selected.

As far as specific anamnestic variables were concerned, significant disparities were found between high and low alcohol consumers with regard to the following: blackouts, "eyeopeners," intoxication, heavy consumption at parties, "more than half a bottle on the same occasion," and subjective classification of personal habits. These positive findings further supported the selection's validity. On the other hand, no differences could be shown in "subjective alcohol problems," violence when drunk, drunken driving, in trouble with the police when drunk, neglecting work, or marriage problems due to alcohol. As to these last variables, a great deal of bias may be expected, as subjects probably responded in accordance with social desirability. Several of the anamnestic variables included are associated with alcoholism. On the other hand, the impression was that many of the high consumers could not really be classified as alcoholics. This might be ascribable to the fact that few questionnaires were probably mailed back by alcoholics in 1967 and to the high concordance regarding consumption of large volumes of alcohol (Kaij 1960, Partanen *et al.* 1966, Jonsson & Nilsson 1968). Thus, such pairs could not have been called in for examination in 1972/73. Furthermore, the strongest significances were consistently obtained for variables pertaining to alcohol volumes consumed (consumption at parties, subjective classification of personal habits, "more than half a bottle on the same occasion") but not for variables commonly considered indicative of alcoholism (blackouts, eyeopeners, intoxication).

As far as physical findings associated with heavy alcohol intake were concerned, a significantly greater proportion of the high consumers were found to have hepatomegaly, tremor, vascular spiders, "paper money" skin and Dupuytren's contracture. Findings indicative of serious liver damage such as patent or suspected jaundice, ascites, and gynecomastia, were not more prevalent

TABLE 9. Distribution of twin pairs by alcohol discordance class according to the 1972/73 interview

Class	Discordance in grams abs. alc./year	MZ	DZ	MZ+DZ	Age MZ+DZ M(SD)
A	> 10,000	5	25	30	54.8 (5.4)
B	5,000—10,000	5	18	23	53.9 (3.9)
C	< 5,000	4	13	17	55.2 (5.0)
	Total	14	56	70	54.6 (4.8)

in the high alcohol group. Results favouring moderate intra pair alcohol discordance were also found with respect to serum enzymes. Although a highly significant difference was noted for SGOT only 2 of the high consumers displayed serum values exceeding 35 U/l, indicating that liver damage would scarcely have been suspected in any of the alcohol consumers in an ordinary medical examination. Similar results were found with respect to SGPT and SALP. The moderate alcohol discordance, is also underlined by the significantly higher SALP and SGT values found among the alcohol positive twins. It is concluded that the results in terms of specific anamnestic variables, physical findings and serum enzymes are in favour of a subject group consisting of moderately alcohol discordant twin pairs and that the data permit the assignment of the twins into a high or a low alcohol group.

GRADING OF ALCOHOL DISCORDANCE

The initial selection of the twin pairs was made on basis of Twin Registry data in accordance with the 1967 questionnaire. Since 6 years had elapsed since the 1967 questionnaire the information in the 1972/73 interviews was regarded as the most adequate basis for discordance classification at the time of the investigation. Thus, this measurement was used for calculation of intra pair differences, and the twins were divided into 3 different discordance classes (Table 9).

CLASS A discordance > 10 000 grams/year
CLASS B discordance 5,000—10,000 grams/year
CLASS C discordance < 5,000 grams/year

SUMMARY

Seventy tentatively alcohol discordant twin pairs were investigated in order to determine the validity of discordance classification. One hundred per cent repeatability was found between the 1967 and the 1972/73 classification with regard to the intra pair high-low relationship Significant differences were obtained between the high and low alcohol group as to the prevalence of the following anamnestic variables Blackouts, eyeopeners, intoxication, heavy consumption at parties, more than half a bottle on the same occasion and subjective classification of personal alcohol habits. In addition, the alcohol positive group showed a significantly increased prevalence of the following physical findings Hepatomegaly tremor vascular spiders, paper money skin and Dupuytren s contracture. Serum enzymes showed significant disparities in SGOT SALP and SGT values, the higher values occurring among high alcohol consumers. It is concluded that the alcohol discordance obtained is valid and permits a separation of twins into a high alcohol group (HAG) and a corresponding low alcohol group (LAG)

Smoking and some pulmonary symptoms

Various epidemiological investigations have shown that morbidity and mortality due to IHD are more prevalent in smokers than in non-smokers. It is assumed that the variables associated with IHD e.g. smoking, may be explained in part by alcohol consumption, as smokers are known to consume more alcohol than non-smokers. The purpose of the work described in this chapter was to determine the extent to which the association between alcohol consumption and cigarette smoking persists when the influence of genetic and early environmental factors is assumed to be minimal. A number of pulmonary symptoms were also studied.

PREVIOUS STUDIES

Smoking and IHD

A possible link between IHD and cigarette smoking was noted in the report of the Royal College of Physicians in 1962. Two years later the U.S. Surgeon-General concluded that male cigarette smokers have a higher death rate from coronary artery disease than non smoking males, but it is less clear that the association has causal significance (U.S. Public Health Service 1964). Reports on the relationship between smoking and IHD have been continuously reviewed ever since, in "The Health Consequences of Smoking" published in 1968 and 1971. These reports concluded that cigarette smokers, particulary men and those in young age groups, were found to have a higher mortality from IHD than non-smokers. The association was found to be stronger for sudden death and fatal myocardial infarction and less definite for angina pectoris.

The statement in the report of the Royal College of Physicians (1971) "that cigarette smoking is an important factor in causing coronary heart disease and that the general avoidance of cigarettes would probably diminish the number of deaths in this condition" is a hypothesis that has yet to be proved. That cigarette smoking aggravates the effect of other coronary risk factors in predisposing to IHD has also been postulated (The National Heart Foundation of New Zealand 1971).

Reviews concerning IHD and associated risk factors have also covered the question of smoking (Epstein 1965, Stamler 1967, 1973, Simborg 1970, Fejfar 1972, Hay 197?). Prospective studies have disclosed an association between smoking and IHD (Kannel *et al* 1968, Carlson & Böttiger 1972, Wilhelmsen *et al* 1973). In the Framingham prospective study (Kannel *et al* 1968) the risk of IHD developing in men was proportional to the number of cigarettes smoked/day but unrelated to the duration of the habit. In a nine year follow-up of 3 168 men in the Stockholm prospective study the incidence of new IHD episodes was found to be strongly related to smoking (Carlson & Böttiger 1972). Wilhelmsen *et al* (1973), using a multiple logistic model, found that smoking was one of the three most powerful predictors of IHD.

Yet the data on morbidity and mortality with respect to IHD in smoking-discordant twin pairs can not be ignored. Lundman (1966), who carried out a clinical investigation of about 200 pairs of smoking-discordant twins failed to find any difference with respect to any IHD manifestations. Neither serum cholesterol nor blood pressure was higher in the smokers than in the non-smoking co-twins. In fact an inverse relationship was found. Liljefors (1970) studying IHD-discordant twin pairs could not demonstrate that the amount of cigarettes smoked differentiated between twins who probably had IHD and those who probably had not.

In a questionnaire study based on the Swedish Twin Registry, Cederlöf *et al* (1966) found an

increased morbidity from angina pectoris among male smokers when the investigational material was regarded as a population sample. However no corresponding increased morbidity could be established when intra-pair comparisons were made between smokers and non-smokers. In continuous investigations of the Swedish Twin Registry (Friberg *et al* 1970 Friberg *et al* 1973) for mortality in twin pairs differing with respect to smoking, an association was found between smoking and mortality including IHD-death, in the DZ but not in the MZ group.

The high incidence of IHD in smokers has drawn attention to the pharmacological effects of acute and prolonged smoking. Lundman (1966) and Rose (1973) have discussed the acute effects of nicotine. By catecholamine release, nicotine has been found to cause an increase in heart rate, contraction rate, stroke volume myocardial irritability and a slight rise in blood pressure and in levels of free fatty acids. Moreover the acute and chronic effects of carbon monoxide have become the subject of increasing interest (U.S. Surgeon-General's Report 1968, 1971 Hay 1972, Rose 1973 Wald *et al* 1973 Arnow 1973) Carbon monoxide reduces the oxygen content of capillary blood and interferes with oxygen release to the myocardium. Astrup (1969 1973) drew attention to the possible atherogenic role of carbon monoxide. An increased endothelial permeability to lipoproteins leading to subendothelial oedema has been shown in animals exposed to high levels of carbon monoxide (Astrup 1969 1973 Kjeldsen *et al* 1972)

Pulmonary findings and symptoms in relation to IHD

An indication that a reduced vital capacity is associated with IHD was initially found in the Framingham Prospective Study (Kannel *et al* 1968) This relationship persisted when the material was subdivided into three classes according to the number of cigarettes smoked. In a 5-year follow-up study of 3,982 men aged 40—59 and initially free from demonstrable IHD Keys *et al* (1972 a) found that low values fo vital capacity were associated with an increased risk of developing IHD However the significant relationship disappeared when comparisons were made age-specific using 5 year classes, but still there was a tendency for a higher rate of IHD development in the quintiles with the lowest vital capacity

Using a multiple logistic model Wilhelmsen *et al* (1973) found that dyspnea, defined according to WHO criteria significantly increased the risk of IHD It was suggested that the link between exertional dyspnea and IHD might indicate that subjects complaining of dyspnea already suffer from some form of cardiovascular or pulmonary dysfunction.

Smoking in relation to heredity

Twin studies by Fisher (1958) and Friberg *et al* (1959) have shown that smoking habits are more similar in monozygotic than in dizygotic twins. These results were confirmed by Cederlöf (1966) who reported concordance with respect to smoking in 57 % of MZ and 50 % of DZ twin pairs. The corresponding figures for concordant non smoking pairs were 23 and 19 % respectively Smoking thus seems to be influenced by genetic factors, although the habit may be regarded as an environmental trait linked to the genetic constitution (Liljefors 1970)

Smoking and alcohol

Smokers are known to consume more alcohol than non smokers (Higgins *et al* 1967 Higgins & Kjeldsberg 1967 Carlsson 1970) In subjects with lung cancer from cigarette smoking, high correlation was also found between smoking and alcohol consumption (Burch & de Pasquale 1967) A comparison of 321 lifelong non-smokers and 279 continuing and former cigarettes smokers in consecutive classes of medical students at Johns Hopkins, disclosed significant differences in levels of coffee and alcohol consumption (Thomas 1973) Friberg *et al* (1973) was able to demonstrate that up to 10% of non-smoking twins were registered in a nationwide alcohol registry as opposed to 30 % of the smokers consuming more than 10 cigarettes/day It has been suggested that part of the reported increased mortality among smokers

TABLE 10 Smoking status in relation to alcohol consumption

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Non smokers	0	2	6	11	6	(8.6)	13	(18.6)
Former smokers	5	8	13	8	18	(25.7)	16	(22.9)
Present smokers	9	4	37	37	46	(65.7)	41	(58.5)

is due to associated factors, such as alcohol consumption, rather than to smoking by itself (Cederlöf 1966 Friberg *et al* 1973)

MATERIAL AND METHODS

The subject group is described in chapter I The twins were interviewed with respect to smoking habits and classified as follows

Non smokers were defined as subjects who had never smoked any form of tobacco.

A former smoker was defined as a subject who denied regular smoking at the time of the investigation but had previously been regular smoker

A present smoker was defined as a subject who smoked regularly at the time of the investigation.

A cigarette smoker was defined as person who smoked cigarettes exclusively

A mixed smoker was defined as a person who smoked both cigarettes, cigars or a pipe.

A cigar/pipe smoker was defined as subject who smoked cigars or pipe exclusively

The cigarette smokers were separated into different groups depending pon the number of cigarettes consumed. 1—4, 5—14 15—24 and ≥ 25 cigarettes/day In the quantitative evaluation of the intra-pair smoking habits this grouping was used to identify the twin showing the highest cigarette consumption.

Pulmonary symptoms were defined as described in Methods (p. 18)

RESULTS

Smoking

Smoking status

A description of the smoking status of the subject group is given in Table 10. All but 6 of the high alcohol consumers (HAG) in the pooled zygosity group had smoked at some time in their lives, i.e. 64 subjects (91.4 %) Present smoking was reported by 46 subjects (65.7 %) in the HAG In the low alcohol group (LAG) there were 13 (18.6 %) non-smokers, i.e. twice as many as in the HAG A total of 57 subjects (81.4 %) in the LAG reported smoking during some period of their lives, 41 of whom (58.5 %) were present smokers. It was pparent that the two groups did not differ markedly with regard to smoking, as shown in an intra-pair comparison. (Table 14)

Type of smoking

A preference for smoking cigarettes was the predominant type of smoking found in the HAG as opposed to cigars or a pipe in the LAG (Table 11) Six HAG subjects (8.6 %) smoked cigars and/or pipe exclusively while the corresponding number for the LAG was 21 subjects (30.0 %) This difference is statistically significant (p

TABLE 11 Type of smoking (former and present smokers combined) in relation to alcohol consumption.

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Cigarette smokers	6	3	25	17	31	(44.3)	20	(28.6)
Mixed smokers	4	4	23	12	27	(38.6)	16	(22.9)
Cigar/pipe smokers	4	5	2	16	6	(8.6)	21	(30.0)

TABLE 12 Number of cigarettes smoked (including former smokers) in relation to alcohol consumption

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Cigarettes only:								
1— 4	1	1	2	6	3	(4.3)	7	(10.0)
5—14	2	1	5	5	7	(10.0)	6	(8.6)
15—24	2	1	12	5	14	(20.0)	6	(8.6)
25—	1	0	6	1	7	(10.0)	1	(1.4)
Cigarettes mixed								
1— 4	1	1	7	1	8	(11.4)	2	(2.9)
5—14	0	2	11	7	11	(15.7)	9	(12.9)
15—24	3	1	4	4	7	(10.0)	5	(7.2)
25—	0	0	1	0	1	(1.4)	0	(0.0)

<0.001) Mixed smoking was reported by 27 HAG subjects (38.6 %) and by 16 LAG subjects (22.9 %). Thirty-one (44.3 %) and 20 (28.6 %) high and low consumers respectively smoked cigarettes only.

Quantity smoked

Table 12, which includes present and former smokers, shows that a high alcohol consumption is also accompanied by a high cigarette consumption. 67.1 % of the HAG reported ≥ 5 cigarettes/day and 38.7 % of the LAG. The corresponding figures for heavy cigarette consumption (≥ 25/day) were 11.4 % and 1.4 % respectively.

The results with respect to present cigarette smoking are found in Table 13. Despite a reduced number of smokers in both groups, the association between high alcohol intake and a high level of cigarette consumption was repeated.

When the number of cigarettes smoked daily was analysed by means of intra-pair comparison, pronounced disparities were also found between high and low alcohol consumers (Table 14). When former smokers were included in the pooled zygosity group 42 HAG subjects displayed a higher level of cigarette consumption than their co-twins. The corresponding number in the LAG was 9 ($p < 0.001$). An estimation of present cigarette

TABLE 13 Number of cigarettes smoked (present smokers) in relation to alcohol consumption

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)			
	High alc.	Low alc.	High alc.	Low alc.	High alc.	%	Low alc.	%
Cigarettes only								
1— 4	1	0	2	5	3	(4.3)	5	(7.2)
5—14		1	5	4	7	(10.0)	5	(7.2)
15—24	0	1	8	1	8	(11.4)	5	(7.2)
25—	1	0	6	1	7	(10.0)	1	(1.4)
Cigarettes, mixed								
1— 4	0	0	5	1	5	(7.2)	1	(1.4)
5—14		0	6	5	6	(8.6)	5	(7.2)
15—24		1	3	3	5	(7.2)	4	(5.7)
25—		0	0	0	0	(0.0)	0	(0.0)

TABLE 14 Frequency of concordance and discordance with respect to smoking in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
HIGH ALC		pos	neg	tot	pos	neg	tot	po	neg	tot
Ever smoked (all types of smoking)	pos	12	2	14	41	9	50	53	11	64
	neg	0	0	0	4	2	6	4	2	6
	tot	12	2	14	45	11	56	57	13	70
Present smokers (all type of smoking)	pos	3	6	9	25	12	37	28	18	46
	neg	1	4	5	12	7	19	13	11	24
	tot	4	10	14	37	19	56	41	29	70
Cigarette level (including former smokers)	pos	4	5	9	8	37***	45	12	42***	54
	neg	2	3	5	7	4	11	9	7	16
	tot	6	8	14	15	41	56	1	49	70
Cigarette level (present smokers)	pos	0	6	6	7	25*	32	7	31**	38
	neg	2	6	8	12	12	24	14	18	32
	tot	2	12	14	19	37	56	21	49	70

* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

consumption gave the same results although less pronounced ($p < 0.01$)

Pulmonary symptoms

Pulmonary symptoms are presented in Table 15 In the pooled zygosity group, cough, defined as regular morning cough, was found in 23 HAG and 10 LAG subjects respectively ($p < 0.01$)

Phlegm was reported by 30 twins in the HAG as opposed to 18 twins in the LAG ($p < 0.05$)

Eight subjects in the HAG had dyspnea defined as grades 3—5 (Grade 3 = shortness of breath when walking with other people of own age on level ground) One subject in the LAG was positive according to this definition ($p < 0.05$) One of the MZ and 2 of the DZ pairs could not be assessed, as one of the twins had decreased myoskeletal locomotive power

Bronchial asthma was found in 26 of the HAG as opposed to 14 of the LAG ($p < 0.01$)

DISCUSSION

Small disparities were found between high and low alcohol consumers with regard to smoking. However, more than twice the number of subjects who never had smoked were found in the low alcohol group. On the other hand, the intra-pair comparison showed a stronger association between the number of cigarettes smoked and the amount of alcohol consumed than the results of the evalua

TABLE 15 Frequen y f on ordanc and di cordanc with pect t me pulmo ary symptom l lat on to al hol con umpti

			LOW ALC								
			MZ			DZ			MZ+DZ		
			pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	Cough	pos	0	4	4	6	13*	19	6	17**	23
		neg	1	9	10	3	34	37	4	43	47
		tot	1	13	14	9	47	56	10	60	70
	Phlegm	pos	1	3	4	10	16*	26	11	19*	30
		neg	2	8	10	5	25	30	7	33	40
		tot	3	11	14	15	41	56	18	52	70
	Dyspnea 1)	pos	0	0	0	0	8*	8	0	8*	8
		neg	0	13	13	1	45	46	1	58	59
		tot	0	13	13	1	53	54	1	66	67
	Bronchial asthma	pos	1	2	3	8	15*	23	9	17**	26
		neg	0	11	11	5	28	33	5	39	44
		tot	1	13	14	13	43	56	14	56	70

1) neg = stages 1–2
pos = stages 3–5

* = $p < 0.05$
** = $p < 0.01$

tion of the smoker/non-smoker dichotomy This was true for MZ and DZ groups. Highly signi ficant differences were found between high and low alcohol subjects in the pooled zygosity group (MZ+DZ) with respect to number of cigarettes smoked when former smoking were included. Similar results were obtained with regard to the present level of cigarette consumption, although less pronounced. That heavy smoking is associated with a high alcohol intake was previously re ported by e.g H gems *et al* (1967) and Thomas (1973) In a study of non-smokers and two groups of heavy smokers, Thomas found that coffee intake and alcohol frequency contributed most heavily to the discrimination. When non-smokers were compared with two groups of light smokers, on the other hand, alcohol frequency did not produce any significant discrimination. The import ance of the level of cigarette consumption to the magnitude of association was also underlined by Friberg *et al* (1973)

As to type of smoking, a larger number of pipe and cigar smokers were found in the alcohol negative group among DZ twins. This would appear to indicate that low consumers of alcohol prefer other types of tobacco than cigarettes. The

association of high alcohol intake in combination with large amounts of cigarettes consumed, as opposed to moderate alcohol consumption and cigar or pipe smoking may be a reflection of different personality types. In this context it is interesting to note that reports by the U.S. Surgeon-General (1968, 1971) Kannel *et al* (1968) and Fejfar (1972) have failed to reveal any link between the smoking of pipes or cigars and IHD morbidity and mortality

An increased prevalence of pulmonary symptoms, such as cough, phlegm, dyspnea and asthma was found in the alcohol-positive group The results were mainly derived from DZ twins but were consistent with results from the MZ group Significantly higher prevalences were noted for all types of symptoms in the DZ and pooled zygosity group. The disparity might have been caused by alcohol, which has been reported to act as a specific risk factor in chronic pulmonary disease owing to the effect of alcohol on mechanisms responsible for the removal of bacteria and inert particles from the lungs (Green & Kass 1965) However a more probable explanation is that the pulmonary symptoms are caused by heavier smoking. Lundman (1966) investigating smoking-discordant twin pairs, found a significantly higher prevalence of respiratory symptoms, such as morning cough, chronic cough and morning phlegm, in the smokers.

SUMMARY

Seventy alcohol discordant twin pairs were investigated with respect to smoking habits and some pulmonary symptoms. Smoking was found to be slightly more prevalent in the high alcohol group. Pipe or cigar smoking turned out to be significantly more frequent among low consumers of alcohol. The numbers of cigarettes smoked showed the most striking differences. A higher level of cigarette smoking was noted in the alcohol positive group. The difference was significant both for present smokers and when former smokers were included. Pulmonary symptoms, such as cough, phlegm, dyspnea and bronchial asthma were more frequently found in subjects with a high alcohol consumption. Significant disparities were found in the prevelance of all these symptoms. It is concluded that moderate longstanding alcohol consumption is accompanied by a high level of cigarette smoking and by the appearance of pulmonary symptoms, even when the influence of genetic and early environmental factors is minimal.

Blood pressure

While the association between hypertension and IHD has been well established, the extent to which this link may be ascribable to external factors is not fully known. It is supposed that variables associated with IHD, such as blood pressure, may be due in part to a high consumption of alcohol. An elevated blood pressure has been found in groups characterized by high alcohol consumption. The purpose of this chapter is to report on the connection between alcohol consumption and blood pressure when the influence of genetic and early environmental factors is assumed to be minimal.

PREVIOUS STUDIES

Blood pressure in relation to IHD

The induction of atherosclerosis experimentally in animals by a high intake of lipids appears to be accelerated by hypertension (Walkerlin *et al* 1957, Heptinstall & Porter 1957). Retrospective studies of humans have revealed a relationship between IHD and hypertension. In such studies, reviewed by Gofman (1959), the incidence of previously occurring hypertension was determined in patients with myocardial infarction. Epidemiological studies have clearly shown elevated blood pressure to be a certain risk factor, indeed, one of the most reliable examined (Epstein 1965, 1968, 1973 b, Semborg 1970, Fejfar 1972, Carlson & Böttiger 197_, Stamler 1973, Wilhelmsen *et al* 1973).

The Framingham prospective study (Kannel *et al* 1971 b) demonstrated that both systolic and diastolic blood pressures were strong predictors of the development of IHD and were independent of cigarette smoking and serum cholesterol levels. The risk was found to be proportional to the magnitude of all indices of arterial pressure, and no "safe" critical level could be identified. The level of casual blood pressure also appeared to be a good predictor of IHD. Discriminant analysis disclosed that the systolic pressure's ability to predict future IHD improved with advancing age. This was not the case for diastolic pressure. Keys *et al* (1971) also found that systolic blood pressure was superior to diastolic blood pressure in the prediction of IHD death or myocardial infarction.

The risks presented by *mild hypertension* have been elucidated (Veterans Administration Cooperative Study Group 1970, Paul 1971, U.S. Public Health Service Hospitals Study Group 1972). In a pooled group of 6 640 men, initially aged 30—59 from 6 different studies, Paul (1971) disclosed that a diastolic blood pressure of 85 to 104 mm Hg, as recorded at an initial examination, had unfavourable implications in terms of total death rate, deaths from IHD and non fatal myocardial infarction. The presence of elevated serum cholesterol and the smoking of cigarettes increased the risk of myocardial infarction and IHD death at essentially all blood pressure levels.

The effects of hypertension *treatment* have been studied e.g. by Mathiesen *et al* (1969) and the Veterans Administration Cooperative Study Group (1967, 1970, 1972). In these studies the incidence of myocardial infarction and sudden death was essentially the same in the treated and control groups.

Blood pressure in relation to heredity

Using twins from a number of twin studies, von Verschuer (1958) found that 53 % of the reported MZ pairs were concordant with respect to hypertension as opposed to 18 % of the DZ pairs. In a study of 200 twin pairs, most of whom less than 15 years of age, Hines *et al* (1957) found that intra-pair differences in both systolic and diastolic blood pressure were less for the MZ than

TABLE 16. Frequency of concordance and discordance with respect to previous diagnosis of hypertension in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	pos	1	1	2	0	11*	11	1	12*	13
	neg	2	10	12	2	43	45	4	53	57
	tot	3	11	14	2	54	56	5	65	70

* =$p < 0.05$

the DZ twins. In a study of smoking-discordant twin pairs Lundman (1966) found a significantly lower intra pair variance in the MZ than in the DZ group with respect to both systolic and diastolic blood pressure. However the levels of significance in this study were considerably greater for the femal group than the male group. In a study of 75 MZ and 84 DZ twin pairs aged 30—40 years, Takkunen (1964) found that systolic blood pressure proved to be subject to genetic influence when intra-pair variance in the two zygosity groups was compared. Diastolic blood pressure on the other hand, was judged to be mainly under environmental influence as no statistical significance was obtained. Deutscher *et al* (1966) observed striking and increasing a resemblance between children and their parents with respect to systolic blood pressure after the age of 40

Mathers *et al* (1961) and Osborne *et al* (1963) investigated 53 pairs of normotensive twins and measured their basal and casual blood pressure. Analysis of the data by comparing intra-pair variance in the MZ and DZ male groups failed to disclose the presence of any sizeable genetic factor However genetic factor was demonstrated for females. Downie *et al* (1968) screening 81 young same-sexed twin pairs, concluded that no evidence of strong genetic component could be detected by means of a single measurement of casual blood pressure. Nor was Liljefors (1970) able to demonstrate any genetic component for systolic or diastolic blood pressures when he analysed the apparently healthy twins in his study

Alcohol and blood pressure

In apparently healthy subjects, *moderate doses of alcohol* are followed by a rather consistent but only transient and slight increase in heart rate, cardiac output and systolic and diastolic blood pressure (Grollman 1942 Holmberg & Mårtens 1955 Stein *et al* 1963 Wallgren & Barry III 1970 Opaleva Stegantseva *et al* 1972) Marbach & Schwertz (1964) observed an increase in cardiac output without any change in blood pressure in humans after alcohol intake indicating a decrease vascular resistance. In experiments with dogs, Webb & Degerli (1965) reported decrease in peripheral resistance and venous pressure after the administration of large doses of alcohol.

The circulatory response to *severe alcohol intoxication* has been studied in animals and humans. Using rabbits Kakolewski & Himwich (1968) found that hypotensive reactions sometimes occurred, even leading to a fatal drop in blood pressure. It was suggested that inhibition of anti-diuretic hormone may contribute to the inability to

TABLE 17 Systolic blood pressure in mm Hg in relation to alcohol consumption

		MZ			DZ			MZ+DZ		
		High alc.	Low alc.	No of pairs	High alc.	Low alc.	No of pairs	High alc.	Low alc.	N of pairs
		M(SD)			M(SD)			M(SD)		
Total	Casual systol	158.1(21.9)	159.6(30.1)	14	158.1(18.7)	148.9(17.6)	56	158.1(19.2)	151.0(20.9)	70
	Basal systol	148.0(21.1)	151.5(3[illegible].3)	14	148.1(16.3)	141.1(17.3)	56	148.0(17.2)	143.4(21.3)	70
Drinkers > 11 000 grams/)	Casual systolic	158.6(21.1)	151.0(30.1)	5	157.2(17.6)	147.1(13.8)	25	157.4(17.9)	147.8(16.3)	30
	Basal	150.1(18.9)	147.0(30.9)	5	147.1(13.6)	140.0(15.3)	25	147.6(14.3)	141.2(18.2)	30

p = < 0.05 p = < 0.01

maintain blood pressure In humans succumbing from alcohol intoxication, hypotension and circulatory failure are prominent features (Edmondson *et al* 1956 Wallgren & Barry III 1970)

Studies of *chronic alcoholics* with and without signs of cardiomyopathy have been reported by e.g. Asokan *et al* (1972) and Spodick *et al* (1972) The patients were studied long after all alcohol had been eliminated from the blood. In these investigations signs of impaired myocardial function were found in the form of low mean cardiac output, elevated mean left ventricular end diastolic pressure and a prolonged pre-ejection period. This was also true of the clinically normal alcoholic subjects. In the study by Spodick *et al* (1972) the clinically normal alcoholic patients had lower systolic and diastolic blood pressures than the control group. The subjects with alcohol induced cardiomyopathy displayed the lowest systolic and diastolic pressures. Alcoholic subjects with cirrhosis of the liver have been said to exhibit a lower incidence of hypertension than a control group (Hall *et al* 1953 Edmondson 1956, Sherlock 1968)

The symptoms associated with *withdrawal* include tremor excessive perspiration, nervousness, weakness, gastric distress, anorexia, hyperreflexia, insomnia and elevated heart rate, body temperature and blood pressure (Cutshall 1965 Wallgren & Barry III 1970 Carlsson 1970) In a study of prolonged drinking and sudden withdrawal, Isbell *et al* (1955) found that symptoms and physical findings began to appear before the blood alcohol level had dropped to zero. Symptoms reached their peak 1 to 2 days after cessation of drinking. No evidence of residual impairment persisted three months after the termination of drinking.

In his study of high blood pressure in men aged 50 Tibblin (1967) found a significant and positive relationship between *alcohol problems* and blood pressure. Alcohol problems in this investigation were defined as at least one entry in the records of the local Temperance Board. * According to Pell & D Alonzo (1973) the incidence of

* Sw "Nykterhetsnämnden

TABLE 18 Frequency of concordance and discordance with respect to casual and basal systolic blood pressure ≥ 140 mm Hg in relation to alcohol consumption

			LOW ALC								
			MZ			DZ			MZ+DZ		
			≥140	<140	tot	≥140	<140	tot	≥140	<140	tot
HIGH ALC	Casual systolic	≥ 140	8	3	11	33	17**	50	41	20**	61
		< 140	1	2	3	5	1	6	6	3	9
		tot	9	5	14	38	18	56	47	23	70
	Basal systolic	≥ 140	6	3	9	23	17*	40	29	20*	49
		< 140	3	2	5	7	9	16	10	11	21
		tot	9	5	14	30	26	56	39	31	70

* = $p < 0.05$

** = $p < 0.01$

hypertension over a five year period was 2.3 times higher for "working alcoholics" than for a matched control group. It was suggested that repeated elevations of the blood pressure after alcohol intake may cause a sustained high blood pressure (D'Alonzo and Pell 1968).

Moderate doses of alcohol in apparently healthy persons tend to produce a transient rise in blood pressure, while severe alcohol intoxication leads to hypotension and circulatory failure. Subjects with alcohol problems have elevated blood pressures, while former alcoholics tend to have low blood pressures. The withdrawal period is generally characterized by elevated blood pressure. The effects of longstanding, moderate alcohol intake on blood pressure have apparently not, so far, been elucidated.

MATERIAL AND METHODS

The subject group appears in chapter I. The twins had their casual and basal blood pressures measured as described in Methods (p. 19). After physical examination, questions regarding previous hypertension and antihypertensive treatment were asked. Previous or present hypertension was accepted when previous or present treatment for high blood pressure was reported by the subject. Two subjects in the high alcohol group (HAG) and the same number in the low alcohol group (LAG) were being treated with antihypertensive agents at the time of the investigation. No twin pair was concordant with respect to present antihypertensive therapy. The twin pairs in which one of the subjects was under treatment were included in the results presented below.

RESULTS

Previous diagnosis of hypertension

An association between alcohol consumption and previous diagnosis of hypertension (Table 16) was found in the pooled zygosity group ($p < 0.05$). Thirteen of the subjects in the HAG versus 5 subjects in the LAG reported previous or present treatment for hypertension. One pair was concordant in this respect. The significant difference between the HAG and LAG was derived from the DZ group. The small number of subjects and the

low prevalence preclude the drawing of any conclusion for the MZ group.

Current level of blood pressure

Systolic blood pressure

A significant association was found between alcohol consumption and systolic blood pressure in the pooled zygosity group (Table 17). Thus, the twin with a high alcohol consumption (HAG) in a pair also had a higher average systolic blood pressure than the twin with a low alcohol consumption (LAG). This result was most pronounced in casual measurement ($p < 0.01$) but was even significant for the basal determination ($p < 0.05$). Mean values amounted to 158.1 and 151.0 mm Hg (casual), 148.0 and 143.4 mm Hg (basal) for the HAG and LAG respectively. Results applied to the DZ group ($p < 0.01$ and < 0.05). Mean values showed no obvious differences in the MZ group.

When the criterion of alcohol discordance was set at > 10 000 g/year (Table 17), systolic mean differences, both casual and basal, were more pronounced, implying a higher mean value for the HAG. This was true for both zygosity groups.

The pressure level limit was set at ≥ 140 mm Hg (Table 18) in evaluating the systolic intra-pair status. The significant connection between alcohol consumption and casual systolic blood pressure was also found in the pooled zygosity group ($p < 0.01$) when this limit was used. Casual systolic pressure ≥ 140 mm Hg was found in 61 HAG and 47 LAG subjects. There were 41 pairs in which both twins had a casual value of ≥ 140 mm Hg. That the association was significant was mainly due to results for the DZ group. Findings were consistent when basal values were analysed ($p < 0.05$).

Diastolic blood pressure

The most obvious finding was an association between alcohol consumption and both casual and basal diastolic blood pressure ($p < 0.001$), as seen in Table 19. This was true for the pooled zygosity

TABLE 19 Diastolic blood pr[illegible] mm Hg [illegible] alcohol [illegible]

		MZ			DZ			MZ+DZ		
		High alc.	Low alc.	N of pairs	High alc.	Low alc.	N of pairs	High alc.	Low alc.	N of pairs
		M(SD)			M(SD)			M(SD)		
Total	Casual diastol	91.6(1[illegible])	93.0(13.7)	11	93.3(9.7)	89.5(9.3)	56	91.6(10.3)	90.2(10.3)	70
	Basal diastol	[illegible](1[illegible])	91.6(13.3)	11	91.8(9.6)	86.5(9.0)	56	91.2(9.8)	87.5(10.1)	70
Discordance [illegible] grams [illegible]	Casual diastol	[illegible].8(8.3)	91.1(12.6)	5	96.3(10.8)	87.8(9.3)	25	95.1(10.8)	88.1(9.7)	30
	Basal diastol	86.8(9.2)	89.1(1[illegible])	5	92.3(10.6)	84.8(9.7)	25	91.1(10.1)	85.5(10.1)	30

p [illegible] 0.05 p < 0.01 p = < 0.001

TABLE 20 Frequency of concordance and discordance with respect to casual and basal diastolic blood pressure ≥ 90 mm Hg in relation to alcohol consumption

			LOW ALC								
			MZ			DZ			tot		
			>90	<90	tot	>90	<90	tot	≥90	<90	tot
HIGH ALC	Casual diastolic	≥ 90	7	(2)	9	32	(15)**	47	39	(17)*	56
		< 90	(3)	2	5	(3)	6	9	(6)	8	14
		tot	10	4	14	35	21	56	45	25	70
	Basal diastolic	≥ 90	5	(1)	6	22	(16)**	38	27	(17)*	44
		< 90	(4)	4	8	(3)	15	18	(7)	19	26
		tot	9	5	14	25	31	56	34	36	70

* = $p < 0.05$
** = $p < 0.01$

group in which mean values for diastolic blood pressure were 94.6 and 90.2 mm Hg (casual) and 91.2 and 87.5 mm Hg (basal) in the HAG and LAG respectively. Results were derived from the DZ group ($p < 0.001$). No such association was found in the MZ group.

Differences in the mean values of the diastolic blood pressures, both casual and basal, appeared to be more pronounced in the pooled zygosity group and among the DZ twins when the alcohol discordance criterion was greatest, > 10 000 g of absolute alcohol/year (Table 19).

In the evaluation of concordance and discordance with respect to casual diastolic blood pressure ≥ 90 mm Hg (Table 20) a significant association ($p < 0.05$) between alcohol consumption and diastolic pressure was again found. Fifty-six and 45 of the HAG and the LAG respectively in the pooled zygosity group were above this limit. There were 39 pairs in which both twins in a pair had a casual value ≥ 90 mm Hg. That the association was significant was mainly due to the results from the DZ group ($p < 0.01$). Differences in basal diastolic pressure in the pooled zygosity group were mainly the same as those obtained for casual measurements ($p < 0.05$).

DISCUSSION

As has been shown, a high alcohol intake is accompanied by elevated blood pressure. Differences in the mean values increased with rising alcohol discordance. This finding could be explained

TABLE 21 Right upper arm circumference in centimeters in relation to alcohol consumption

MZ (N=14 pairs) High alc.	MZ Low alc.	DZ (N=56 pairs) High alc.	DZ Low alc.	MZ+DZ (N=70 pairs) High alc.	MZ+DZ Low alc.
M(SD)		M(SD)		M(SD)	
29.0(2.5)	29.8(1.9)	28.7(2.4)	28.7(2.7)	28.7(2.4)	28.9(2.6)

by the presence of alcohol in blood (discussed e.g by Holmberg & Mårtens 1955 Wallgren & Barry III 1970) However this was so in only one of the 140 subjects examined. Withdrawal also gives rise to elevated blood pressure (Isbell *et al* 1955 Cutshall 1965) However the subjects were ignorant of the purpose of the study and so it may be assumed that their drinking habits had not changed in the weeks preceding the examination. On the other hand, they were ordered to fast from the evening prior to the day of examination. Even if some subjects with a high alcohol intake had stopped drinking because of travelling, etc., more than 24 hours prior to examination, sustained elevated catecholamine levels might explain the elevated blood pressure as shown by Brohult *et al* (1970)

The finding of higher blood pressure levels in the alcohol positive group agreed with the history of previous or present hypertension given. This association is thus confirmed by other in principle, independent investigators and other periods of time.

How an increased upper arm circumference results in an elevated auscultatory blood pressure has been discussed by Tibblin (1967) and Rose & Blackburn (1968) This could not explain elevated systolic and diastolic blood pressure found in the DZ group, since mean values for the upper arm circumference were identical in the high and low alcohol groups (Table 21) However a lower right upper arm circumference was found for the high alcohol consumers in the MZ group. This difference was not significant using a paired "t -test but was probably not random. When the MZ pairs were tested using the McNemar non-parametric test (Siegel 1956) a significantly larger number of the alcohol-positive twins had circumferences less than their co-twins. Apart from genetic considerations, this might explain blood pressure results in the MZ group.

It must be emphasized that the number of subjects in the monozygotic group, in which no association was found, was small The selection of MZ twin pairs exhibiting discordance > 5 000 g of absolute alcohol/year produced 10 pairs. The high alcohol consuming twins in 7 pairs displayed a higher casual diastolic blood pressure than their co-twins. The reverse was found in 3 pairs. This might mean that the association between alcohol consumption and blood pressure may persist even when the influence of genetic factors is minimal.

SUMMARY

The blood pressures in seventy alcohol-discordant twin pairs were examined. In the pooled zygosity group (MZ+DZ) a significantly greater proportion of the high consumers had a previous history of hypertension than the low alcohol consumers. A significant association was also found between alcohol consumption and the current blood pressures, both systolic and diastolic. The findings were independent of whether measurements were casual or basal. These results point to an association between moderate, longstanding alcohol consumption and elevated blood pressure when the influence of genetic and early environmental factors is assumed to be minimal.

Weight and skinfold thickness

A number of epidemiological investigations have shown a link between obesity and morbidity and mortality due to IHD. However, obesity appears to be a factor of limited importance. It is supposed that the factors associated with IHD, e.g. over-weight, may be partly explained by a high alcohol consumption. There is little information about the relationship between alcohol consumption and weight. The purpose of this chapter is to elucidate the relationship between longstanding alcohol consumption and weight (absolute and relative) and skinfold thickness when the influence of genetic and early environmental factors is minimal.

PREVIOUS STUDIES

Weight and IHD

Long before the current preoccupation with risk factors for IHD, American life insurance companies were able to demonstrate increased mortality due to atherosclerotic disease in over-weight subjects (Dublin 1930). Master *et al* (1953) found an increased incidence of overweight in men for all forms of IHD as compared to a control group. Several investigations have disclosed a relationship between body build, "mesomorphy" and IHD (e.g. Gertler & White 1954, Walker & Gregoratus 1967). However the heavy weight in these studies was not a manifestation of obesity but mesomorphic body build. The Framingham prospective study (Kannel *et al* 1967a) comprising more than 5,000 subjects aged 30—62 years at entry revealed an excess risk for angina pectoris and sudden death for obese men both with and without elevated blood pressure and serum cholesterol. Thus, these results indicate an independent contribution by obesity to the rate of development of some manifestations of IHD. According to Epstein (1965) there is little doubt that obesity per se is a less potent risk factor than such a factor as serum cholesterol or the level of blood pressure. In his review Simborg (1970) concluded that overweight subjects "have an increased risk of angina pectoris and sudden death not due to myocardial infarction.

In the seven-year follow-up of Evans County (Heyden *et al* 1971) in which smokers were compared to non-smokers, the risk of developing IHD did not increase from the leanest to the most obese non-smokers. However a link between over-weight and IHD was apparent in smokers. Keys *et al* (1972b) studying more than 10 000 middle-aged men with multivariate analysis (multiple logistic equation) was able to show that no measure of relative weight or obesity made any significant contribution to future IHD irrespective of age, blood pressure, serum cholesterol and smoking.

Weight and heredity

In a study of male twins aged 30—40 years, i.e. 75 MZ and 84 DZ twins, Takkunen (1964) found that skeletal dimensions were chiefly influenced by heredity. Both upper arm and subscapular skinfold thickness were poor measures of hereditary influence and were almost exclusively dependent on environmental factors. Weight was found to be dependent on both hereditary and environmental factors. Similar results were obtained by Lundman (1966) and Liljefors (1970) for weight.

Weight and alcohol consumption

There is little information for humans concerning weight in relation to alcohol consumption. In a study of healthy and gainfully employed middle-aged men, it was found that the heaviest men consumed the largest quantities of alcohol (Tofler *et al* 1969) and weight gain was correlated to

heavy current beer and alcohol consumption. In a comparison of overweight in alcoholics and controls in an American company D Alonzo and Pell (1968) found that the proportion of drinkers and controls who were 20 % or more over their ideal weight was about the same. However a significantly larger percentage of drinkers were below ideal weight (< 0.9) that is 18 2 % as compared to 11 4 % Eriksson (1969) found a lower body weight in heavy-drinking rat strains than in low consumption strains. The heavy-drinking strain also had a larger intake of solid food, suggesting a higher level of general metabolism for the heavy drinkers.

MATERIAL AND METHODS

The subject group appears in chapter I The twins had their weight and skinfold thickness measured as described in Methods (p 19) Their relative weight was determined according to the following equation

weight (kg)

0 9 (height, cm-100)

Overweight was defined as a quotient ≥ 1 20.

RESULTS

Absolute and relative weight

The main finding of this study was the lack of any link between absolute weight and alcohol consumption (Table 22) The difference in mean values in the highest discordance class (A) for absolute weight amounted to 73 4 and 70.0 kg in the HAG and LAG respectively

A analysis of relative weight (Table 23) failed to disclose any significant disparities between the high and low alcohol consumers. In the highest discordance class (A) however an increased number of alcohol positive twins had a relative weight quotient ≥ 1 20

Skinfold thickness

A significantly thicker layer of subcutaneous fat was found in the triceps area (Table 24) the highest discordance class (A) for the high alcohol consumers (p 0 05) in the pooled zygosity

TABLE 2 Absolute weight kg ... to alcohol consumption

Class	Discordance in abs alc/year (grams)	MZ High alc M(SD)	MZ Low alc M(SD)	MZ No of pairs	DZ High alc M(SD)	DZ Low alc M(SD)	DZ No of pairs	MZ+DZ High alc M(SD)	MZ+DZ Low alc M(SD)	MZ+DZ No of pairs
A	> 10 000	71 4(20 6)	70.4(14 5)	5	73.8(11 1)	69 9(10 9)	25	73 4(12 7)	70 0(11.3)	30
B	5 000—10,000	79 0(6 7)	79 1(12 0)	5	71 3(12.1)	72 6(10 4)	18	75 4(11 5)	71.1(10 8)	23
C	< 5,000	68.5(8 6)	71 0(11 1)	4	71 1(12.5)	77 4(14.4)	13	72 8(11 6)	76 6(13 5)	17
	Total	73 3(13 5)	71 6(1 6)	14	71 0(11 7)	72 5(11.8)	56	3 9(11 9)	72 9(11 9)	70

TABLE 23 Frequency of concordance and discordance with respect to relative weight ≥ 1.20 in relation to alcohol consumption

			LOW ALC								
			MZ			DZ			MZ+DZ		
			≥1 20	<1 20	tot	≥1 20	<1 20	tot	≥1 20	<1 20	tot
HIGH ALC	Total	≥ 1 20	4	1	5	4	10	14	8	11	19
		< 1 20	2	7	9	8	34	42	10	41	51
		tot	6	8	14	12	44	56	18	52	70
	Discordance > 10 000 g/year	≥ 1 20	2	0	2	1	7	8	3	7	10
		< 1 20	1	2	3	2	15	17	3	17	20
		tot	3	2	5	3	22	25	6	24	30

group. This finding was not noted in the two lower discordance classes (B and C).

No significant disparities were found in the subscapular area (Table 25) in high and low consumers of alcohol.

DISCUSSION

The present study found no significant positive association between alcohol consumption and increased absolute or relative weight. Such an hypothesis has received but little support in previous reports, due, perhaps to a failure to report negative findings.

Differing behaviour regarding the type and frequency of alcohol consumption may have different effects on weight. German beer drinkers, for instance, are known to be obese. In a study of 8 young (22—26 years) healthy volunteers, prolonged beer administration, corresponding to 63 grams of absolute alcohol/day increased body weight in all cases but one during an experimental period of 5 weeks (Belfrage *et al* 1973). On the other hand, subjects with a heavy consumption of hard liquor and a poor diet may be lean. When heavy alcohol consumption is accompanied by somatic complications, such as gastric ulcer, pancreatitis and malabsorption (Carlsson 1970, Wallgren & Barry III 1970) affected subjects often have a lower-than-average weight.

TABLE 24 Skinfold thickness in triceps area (mm) in relation to alcohol consumption

Class	Discordance in abs alc / year (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No of pairs
A	> 10,000	8.3(3.5)	8.6(3.9)	5	9.4(4.8)	7.7(3.0)	25	9.2(4.5)	7.8(3.1)	30
B	5 000—10 000	10.6(3.0)	12.9(11.3)	5	8.5(2.8)	8.5(2.7)	18	8.9(2.9)	9.5(5.7)	23
C	< 5 000	8.4(2.2)	10.6(3.2)	4	9.1(2.9)	9.8(.5)	13	8.9(2.7)	10.0(2.6)	17
	Total	9.0(2.9)	10.7(7.1)	14	9.0(3.8)	8.4(2.9)	56	9.0(3.6)	8.9(4.1)	70

= p < 0.05

TABLE 25 Skinfold thickness in subscapular area (mm) in relation to alcohol consumption

Class	Discordance in abs. alc/years (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No. of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No. of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No. of pairs
A	> 10,000	16.0(5.4)	15.2(2.1)	5	17.4(6.0)	15.3(7.1)	25	17.2(5.8)	15.3(6.5)	30
B	5 000—10,000	23.6(9.0)	21.9(9.5)	5	16.5(6.5)	15.7(4.4)	18	18.0(7.6)	17.0(6.2)	23
C	< 5,000	13.1(4.8)	17.8(5.5)	4	17.1(5.6)	19.9(6.6)	13	16.2(5.6)	19.4(6.0)	17
	Total	17.9(7.8)	18.5(6.5)	14	17.0(6.0)	16.5(6.4)	56	17.2(6.3)	16.9(6.4)	70

Despite the absence of any link between alcohol consumption and weight in the present study a positive and significant relationship was noted between subcutaneous fat thickness in the triceps area and alcohol intake in the highest discordance class. A similar trend with respect to the subscapular area was also found. The fact that skinfold thickness only was of significance is in accordance with the assumption that this parameter has a more exclusively environmental origin (Takkunen 1964, Liljefors 1970). However results were too heterogenous to support any obvious relationship between obesity and unspecified alcohol consumption, at least as long as the influence of genetic and early environmental factors was assumed to be minimal.

SUMMARY

Seventy alcohol-discordant twin pairs were examined with respect to absolute and relative weight and skinfold thickness. No significant link was found between alcohol consumption and any measure of weight. The alcohol-positive twins in the highest discordance class of the pooled zygosity group (MZ+DZ) had a significantly thicker layer of subcutaneous fat in the triceps area than their low consumption co-twins. No differences were found for the subscapular area. It is concluded that no obvious relationship between unspecified alcohol consumption and obesity can be demonstrated when the influence of genetic and early environmental factors is expected to be minimized.

An association between various disorders of lipid metabolism and IHD has been established. It is assumed that variables associated with IHD such as serum lipid levels, may be explained to some extent by alcohol habits. In clinical experiments, acute and prolonged alcohol administration to healthy subjects has been reported to increase the level of serum lipids. Intense lipidaemia has also been recognized in alcoholics. The purpose of this chapter is to consider the possible action of prolonged, moderate alcohol consumption on the amounts of lipoproteins and lipids in serum when the influence of constitutional factors is assumed to be minimal.

PREVIOUS STUDIES

Cholesterol and IHD

In investigations using laboratory animals the severity of atherosclerotic lesions has been found to increase with increasing cholesterol levels (Malmros & Wigand 1959). Clinical studies have shown a significantly increased number of subjects with elevated serum cholesterol in groups suffering from IHD as compared to control groups (Biörck *et al.* 1957 b, Arnow *et al.* 1973 Goldstein *et al.* 1973). This applies especially to younger age groups.

The close connection between elevated cholesterol levels and IHD is also apparent from a number of reviews and prospective epidemiological studies (Epstein 1965 Samborg 1970, Fejfar 1972 Stamler 1973). A linear relationship was found between cholesterol level and IHD when incidence data from studies in 7 countries were compared (Key 1970). The Framingham prospective study (Kannel *et al.* 1971 a) noted a stronger positive association between IHD and serum cholesterol in younger persons than in older persons. Furthermore, the risk of each clinical manifestation of IHD proved proportional to the antecedent cholesterol level. The Stockholm prospective study (Carlson & Böttiger 1972) found that the IHD rate was directly proportional to fasting concentrations of serum cholesterol in subjects under 60 years of age, but not in an older age-group. Using a multiple logistic model, Wilhelmsen *et al.* (1973) found that the presence of high serum cholesterol levels as compared to 8 other variables was the most reliable, independent, discriminating factor in the development of non fatal myocardial infarction and acute IHD death.

Triglycerides and IHD

The literature on serum triglycerides and IHD is not very extensive but suggests a link between a high level for serum triglycerides and IHD. Albrink & Man (1959) reported that serum triglyceride levels were more often elevated in patients with IHD than in healthy subjects. Carlson (1960) found significantly elevated levels of serum triglycerides in men under 50 years with infarction. In a study of lipid levels in 500 consecutively 3-month survivors of myocardial infarction, Goldstein *et al.* (1973) were able to demonstrate that 31 % had hyperlipidaemia lipid abnormalities among the males were most commonly found in patients under 40. Furthermore, these authors showed that elevated serum triglycerides, with or without concomitant elevation of serum cholesterol, was three times more common in survivors than a high cholesterol level alone. In the Stockholm prospective study of slightly more than 3 000 men (Carlson & Böttiger 1972) the rate of IHD was directly proportional to increasing fasting concentrations of serum triglycerides. Both serum triglycerides and serum cholesterol were found to be independent risk factors in IHD but

significant differences for both lipids could only be shown for subjects under 60 years of age.

Serum lipoproteins and IHD

Several investigators have emphasized that it is inadequate to classify basic serum lipoprotein abnormalities simply by reference to elevated serum cholesterol or serum triglycerides. Thus, Fredrickson & Lees (1965) suggested a hyperlipoproteinaemia classification system, which was modified in 1970 in accordance with Beumont *et al.* This system provides a method of systematically classifying the lipoproteins. The hyperlipoproteinaemias of major interest in connection with IHD are Types II A, II B, III and IV (Gustafson 1973). Type II A denotes an increase in serum cholesterol. This is seen as a beta-lipoprotein pattern in lipid electrophoresis. In types II B and III there is an increase both in serum cholesterol and a rise in serum triglycerides presenting as a mixture of beta- and pre-beta patterns in electrophoresis. Type IV denotes an increased triglyceride level, a moderate elevation of serum cholesterol values and a pre-beta pattern.

Blood lipids in relation to heredity

Several investigators have disclosed a varying degree of genetic influence on serum cholesterol and phospholipids in man but there is little data on serum triglycerides and lipoproteins.

The importance of genetic factors on serum cholesterol levels has been firmly established (Wilkinson *et al.* 1948) in respect to primary hypercholesterolaemia (II A). In a study of 34 twin pairs, Meyer (1962) found a significantly smaller intra pair variance for serum cholesterol in MZ than DZ pairs living together thus indicating a genetic influence. On the other hand, twins living apart displayed variances exceeding those displayed by twins living together thereby demonstrating the importance of environmental factors as well. Similar results were reported by Osborn *et al.* (1959) McDonough *et al.* (1962) Jensen *et al.* (1965) and Rifkind *et al.* (1968). Lundman (1966) found an intra pair variance for serum cholesterol which was lower in MZ than in DZ twins but not significantly so. Liljefors (1970) revealed an apparent genetic influence on cholesterol levels in terms of intra pair variance comparison. Heiberg (1973) also found an obvious genetic influence on cholesterol levels, and the use of H statistics suggested that the variation for men was genetically determined to 84 %.

Lundman (1966) found lower intra-pair variances for MZ than for DZ twins with respect to serum triglycerides. However significant differences were only found for females. The subject group used by Liljefors (1970) consisted exclusively of males, and he was unable to show any definite genetic effect on the triglyceride level, which varied to the same extent within pairs, irrespective of zygosity. Nor could Heiberg (1973) find any formal evidence for genetic determination of serum triglyceride levels.

Alcohol and blood lipids

Prolonged, moderate administration of alcohol to 8 young volunteers only produced minor non-significant elevations of serum cholesterol levels (Berg & Johansson 1973). Adelson & Keys (1963) found that a moderate-to-high habitual intake of alcohol by 123 business and professional men was associated with elevated serum cholesterol values. Serum cholesterol in 7 chronic alcoholics was found to increase during prolonged administration of alcohol (Lieber *et al.* 1963). Experimental studies using animals and reviewed by e.g. Hirst *et al.* (1965) and Wallgren & Barry III (1970) have generally disclosed elevation of serum cholesterol during prolonged alcohol administration. Lefevre *et al.* (1972) found an increase in serum cholesterol during alcohol feeding of rats and concluded that this was linked to both enhanced cholesterogenesis and decreased excretion of bile acids.

A large increase in serum triglycerides is often seen in subjects with alcohol abuse (Lieber *et al.* 1963. Losowsky *et al.* 1963. Chait *et al.* 1972, Sirtory *et al.* 1972). Even a large single dose (Nestel *et al.* 1965. Brohult *et al.* 1970) or prolonged moderate alcohol intake (Berg & Johansson 1973) in apparently healty subjects has been found to be

TABLE 26. Serum cholesterol in relation to alcohol consumption

Class	Discordance in abs. alc./year (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No. of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No. of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No. of pairs
A	> 10,000	215(38)	279(75)	5	234(45)	223(43)	25	231(44)	225(48)	38
B	5,000—10,000	241(25)	262(68)	5	245(47)	238(55)	18	244(43)	243(57)	23
C	< 5,000	229(47)	230(33)	4	253(48)	238(55)	13	248(47)	236(43)	17
	Total	228(36)	241(61)	14	242(46)	232(48)	56	239(44)	33(50)	70

accompanied by an increase in serum triglycerides. Mendelson & Mello (1973) found dramatic increases in serum triglycerides in alcoholic subjects with evidence of a type IV hyperlipoproteinaemia as compared to normal controls following alcohol ingestion. This result and the findings of Losowsky *et al.* (1963) suggest that the triglyceride response to alcohol intake is more pronounced in the presence of a preexisting lipo-protein derangement.

Lipoprotein patterns in alcoholics have been investigated by Johansson & Laurell (1969) who found an increase in alpha-lipoproteins. This finding was in accordance with Berg & Johansson (1973) who also found a slight but significant increase in alpha-lipoprotein levels in apparently healthy volunteers during prolonged moderate alcohol consumption. In all cases the increase was within normal limits. These studies showed no abnormalities in the other serum lipoproteins.

MATERIAL AND METHODS

The subject group is described in chapter I.

The twin pairs were examined with respect to serum cholesterol and serum triglycerides after an overnight fast. Lipoprotein patterns were determined for all twin pairs except the first 9. The methods applied are described on page 21.

RESULTS

Serum cholesterol

The mean values for serum cholesterol in the pooled zygosity group (Table 26) were not significantly different in the high and low consumers of alcohol. These were 239 and 233 mg/100 ml in the HAG and LAG respectively. A higher mean cholesterol level was consistently found in the HAG in the three discordance classes (A-C). Sixty-one and 51 subjects in the HAG and LAG respectively displayed serum cholesterol values > 200 mg/100 ml (Table 27). There was concordance in 47 pairs on the basis of this criterion. In the 18 twin pairs in which one of the members had a serum cholesterol value > 200 mg/100 ml, the value in 14 pairs applied to the high consumer group ($p < 0.05$). An identical tendency was found when the limit for serum cholesterol was set at 250 mg/100 ml (Table 27).

Serum triglycerides

The high and low consumers in the pooled zygosity group showed the same mean value of triglycerides, i.e. 147 mg/100 ml (Table 28). In the highest discordance class (A) however an association between alcohol consumption and the level of triglycerides was suggested as mean values amounted to 140 and 122 mg/100 ml in the HAG and LAG respectively. *However this was not significant.* Furthermore, no such association could be found in the other discordance classes (B and C). Twenty seven and 24 subjects in the HAG and LAG respectively exceeded the 150 mg/100 ml limit when intra pair comparison was made of the level of triglycerides (Table 29). The result was consistent even when the limit was set at > 200 mg/100 ml. Thus, these findings suggest that there is no association between habitual alcohol consumption and the fasting triglyceride level.

TABLE 27 Frequency of concordance and discordance with respect to serum cholesterol level > 200 mg/100 ml and > 250 mg/100 ml in relation to alcohol consumption

LOW ALC	MZ			DZ			MZ+DZ		
	>200	≤200	tot	>200	≤200	tot	>200	≤200	tot
> 200	9	3	12	38	11	49	47	14*	61
≤ 200	0	2	2	4	3	7	4	5	9
tot	9	5	14	42	14	56	51	19	70

HIGH ALC	MZ			DZ			MZ+DZ		
	>250	≤250	tot	>250	≤250	tot	>250	≤250	tot
> 250	2	2	4	9	11*	20	11	13	24
≤ 250	2	8	10	3	33	36	5	41	46
tot	4	10	14	12	44	56	16	54	70

* = $p < 0.05$

Serum lipoproteins

Negligible differences were found in lipoprotein levels. If a ≥ 60 % limit for a pathological beta lipoprotein level was used, 23 subjects in the HAG and 1 subjects in the LAG were found to exceed this level (Table 30). The same was true for the pre-beta lipoproteins as 11 and 10 subjects in the HAG and LAG respectively exceeded the 5 % value used as a limit for a pathological pre beta lipoprotein level (Table 31). The results were heterogenous with respect to alpha-lipoprotein and also failed to show any differences between high and low alcohol consumers.

DISCUSSION

A significant association of cholesterol levels exceeding 200 mg/100 ml in the pooled zygosity group and alcohol consumption was demonstrated. This relationship was not especially reliable as no significance was disclosed when intra pair differ

TABLE 28 Serum triglyceride in relation to alcohol consumption

Class	Discordance in abs alc year (grams)	MZ High alc M(SD)	MZ Low alc M(SD)	MZ N of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ N of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ N of pairs
A	10 000	156(4)	140(3)	5	13 (93)	118(41)	25	110(89)	122(10)	30
B	5 000—1 000	119(18)	115(79)	5	165(100)	193(85)	18	155(90)	1 6(85)	3
C	< 5 000	150(5)	161(35)	4	155(61)	145( )	13	149(56)	151(69)	17
	Total	135(49)	13 (37)	11	150(9)	149(1)	56	147(82)	14 (68)	70

TABLE 29 Frequency of concordance and discordance with respect to triglyceride level > 150 mg/100 ml and > 200 mg/100 ml in relation to alcohol consumption.

	LOW ALC								
	MZ			DZ			MZ+DZ		
	>150	≤150	tot	>150	≤150	tot	>150	≤150	tot
> 150	3	1	4	11	12	23	14	13	27
≤ 150	1	9	10	9	24	33	10	33	43
tot	4	10	14	20	36	56	24	46	70
HIGH ALC	>200	≤200	tot	>200	≤200	tot	>200	≤200	tot
> 200	0	2	2	4	7	11	4	9	13
≤ 200	1	11	12	9	36	45	10	47	57
tot	1	13	14	13	43	56	14	56	70

ences were assessed using the paired "t" test. However, in view of the fact that the high alcohol consumers were to a significant extent heavier smokers (chapter IV), a finding in accordance with earlier studies (e.g. Friberg *et al.* 1973), the results here shown are not without interest. Lundman (1966) was able to demonstrate a consistent reduction of serum cholesterol in smoking subjects in his earlier twin study of smoking discordant twins. Furthermore, the link between alcohol consumption and serum cholesterol might have been more pronounced if casual levels had been investigated. In earlier studies, during and immediately after a very large alcohol intake, elevated serum cholesterol levels have been demonstrated (Lieber *et al* 1963, Losowsky *et al* 1963). A normalization of serum cholesterol levels 1—2 days after the cessation of alcohol intake was generally found in these reports. In the present subject group, it is presumed that an overwhelming ma-

TABLE 30 Frequency of concordance and discordance with respect to beta lipoprotein level ≥ 60 per cent in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		≥60	<60	tot	≥60	<60	tot	≥60	<60	tot
HIGH ALC	≥60	1	4	5	9	9	18	10	13	23
	<60	3	5	8	11	19	30	14	24	38
	tot	4	9	13	20	28	48	24	37	61

TABLE 31 Frequency f oncordant and discordance ith re pect t pre-beta lip protein l el ≥ 5 per cent in relation t al ohol mption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		≥25	<25	tot	≥25	<25	tot	≥25	<25	tot
HIGH ALC	≥ 5	1	(2)	3	4	(4)	8	5	(6)	11
	<25	(1)	9	10	(4)	36	40	(5)	45	50
	tot	2	11	13	8	40	48	10	51	61

jority of the high alcohol consumers were abstinent for 12—24h prior to the drawing of blood samples. Despite the fact that many subjects in the alcohol positive group might be exposed to repeated elevations of serum cholesterol, a high level was not apparently maintained after abstinence.

Accord g to the present study moderate alcohol consumption does not appear to significantly alter the level of triglycerides when measurements are made d ring a period of abstinence. However the immediate effect of alcohol on serum triglycerides, as reported in alcoholic and healthy subjects (Lieber *et al* 1963 Losowsky *et al* 1963 Brohult *t al* 1966 Berg & Johansson 1973) was also demonstrated in the present study The one subject who had measurable blood alcohol (1 4 /₀₀) and who belonged to the alcohol-positive group had the highest triglyceride value in all determinations, i.e. 500 mg/100 ml This subject, who also belonged to the h ghest DZ twin discordance class (A) was to some extent responsible for the tendency towards an elevated trigly eride level for the high alcohol consumers. In two tw n pairs in the subject group whose fasting serum lipids were followed for five days, marked fluctuations especially in serum triglycerides were noted for the alcohol positive twins (Fig. 2)

No obvious l poprotei d fferences were found between h gh and low alcohol consumers. These findings may be d e g to genetic influences, as claimed by Heiberg (19 3) and others or to the fact that the high onsumers were abst nent the day or days before investigation. It seems reasonable to assume that a fasting lipid value does not give a true picture in many cases of the lipid levels of a subject with a high habitual alcohol consumption. Hypertriglyceridaemia two or three times a week with a concomitant elevation in serum cho-

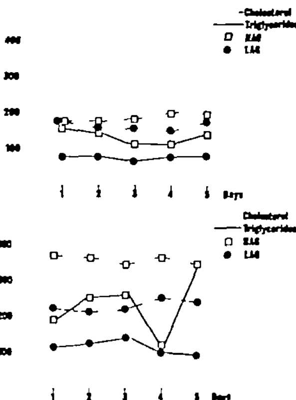


Fig Serum hol sterol and serum triglycerid les l in mg 100 ml tak f ur g stat dar g 5 on at day 2 t in pair (DZ pair 33 and 34 year old)

lesterol may be overlooked if the subject remains abstinent one or two days before blood samples are drawn. A quick normalization of the triglyceride level has been reported in alcoholics while in hospital (Chait *et al.* 1972). Casual lipid values have been analysed in epidemiological studies of serum lipids and IHD, such as the Framingham prospective study (Kannel *et al.* 1971 a). The results from casual measurements could imply that part of the association between serum lipids and IHD is explained by alcohol consumption.

SUMMARY

Seventy alcohol-discordant twin pairs were investigated with respect to serum cholesterol, triglyceride level and lipoprotein patterns. The data suggest a link between longstanding, moderate alcohol consumption and elevated serum cholesterol. Significantly higher numbers of the high alcohol consumers in the pooled zygosity group (MZ+DZ) displayed serum cholesterol values exceeding 200 mg/100 ml. No obvious disparities were revealed in either serum triglycerides or lipoprotein patterns. It is suggested that longstanding, moderate alcohol consumption is not apparently linked to elevated fasting serum lipids when the influence of genetic and early environmental factors is minimal.

Blood glucose and intravenous glucose tolerance

The association between IHD and various disorders of glucose metabolism is well known. It is assumed that variables associated with IHD such as the blood glucose level and intravenous glucose tolerance (IVG) may be due in part to a high alcohol intake. Alcohol administration in humans has been reported to cause a transient elevation in blood glucose levels, and an abnormal glucose metabolism has been shown to characterize "alcoholic subjects. The purpose of this chapter is to analyse the possible effects of a longstanding alcohol consumption on fasting blood glucose levels and intravenous glucose tolerance when the influence of hereditary and early environmental factors is minimal.

PREVIOUS STUDIES

Overt diabetes and IHD

An overrepresentation of diabetes mellitus in subjects with myocardial infarction has been shown by e.g. Eckerström (1951) Linden (1952) Sievers *et al* (1961) and Wahlberg (1962). Entmacher *et al* (1964) was able to show that the incidence of coronary deaths in diabetics trebled from 1922 to 19(Furthermore the long-term prognosis for diabetic survivors of myocardial infarction was less favourable than for non-diabetics, according to Biorck *et al* (1957 a) Linden (1962) Sievers (19 3) Partamian & Bradley (1965) Wahlberg (1966) and Paasikivi (1970). In a recent study however Helmers (1973) was unable to demonstrate that the prognosis following myocardial infarction w any worse for diabetics

Impaired glucose tolerance and IHD

The relationship between milder disorders of glucose metabolism and IHD has been the subject of great interest in the last ten years (Epstein 1965 1969 1973 a, b). A number of studies have disclosed a high prevalence of decreased intravenous and oral glucose tolerance in patients with manifest IHD (Wahlberg 1966 Paasikivi 1970) Most investigations of this kind have been based on survivors of myocardial infarction or patients with otherwise clearcut clinical evidence of IHD (for references, see Pyörälä 1973)

Additional information about the relationship between impaired glucose tolerance and IHD has been obtained from population studies. The results of oral glucose tolerance tests were analyzed in these studies in relation to clinical and ECG manifestations of IHD Studies of this kind have been performed by e.g. Keen *et al* (1965) Ostrander *et al* (1965) Welborn *et al* (1969) and Pyörälä (1973) With the exception of the latter these studies, after excluding patients with overt diabetes, disclosed a correlation between hyperglycaemia and IHD In Pyörälä's prospective study (1973) of 1 269 Finnish policemen, no difinite relationship was found between hyperglycaemia and the prevalence of angina pectoris or possible infarction according to Rose's questionnaire (1964) However ECG abnormalities displayed an increased prevalence with rising blood glucose levels in the 30—49 year age group.

Hyperglycaemia in relation to heredity

Diabetes mellitus is generally accepted as being, at least to some extent, an inherited disease (Cerasi 1967 Marble *et al* 1971) as shown by an increased concordance for the disease in identical twins and an increased prevalence in the offspring of diabetics.

Hyperglycaemia and glycosuria are late signs of the disorder and the overt phase of the disease is preceded by latent or silent stages revealed by glucose tolerance tests (Cerasi 1967) Then Berg (1938) investigated 133 initially diabetes-discor

TABLE 32. Fasting blood glucose in mg/100 ml in relation to alcohol consumption

Class	Discordance in abs. alc./year (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No. of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No. of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No. of pairs
A	> 10,000	95(13)	88(18)	5	82(16)	79(13)	25	84(16)	81(14)	30
B	5 000—10,000	84(14)	83(11)	5	81(21)	80(7)	18	84(19)	81(8)	23
C	< 5,000	71(8)	75(10)	4	89(20)	78(9)	13	85(19)	77(9)	17
	Total	84(15)	82(14)	14	84(19)	79(10)	56	84(18)	80(11)	70

$* = p < 0.05$

dant twin pairs for evidence of latent diabetes in the clinically normal co-twin. She found that diabetes or latent diabetes was present in both members in 65 % of the MZ and 20 % of the DZ. Furthermore, both twins had diabetes in all MZ pairs more than 43 years of age. Gottlieb & Root (1968) studied 104 twin pairs from the Joslin Clinic and found a high concordance for overt diabetes in MZ twins in which the proband was 40 or more at the time of diagnosis. Similar results have been reported by Harvald & Hauge (1963) who found concordance for overt diabetes in 60 % of MZ versus 13 % of DZ same sexed twins. Cerasi & Luft (1967) studied MZ twin pairs consisting of one diabetic and one member with a normal glucose tolerance. A striking similarity was found between their low insulin responses after glucose infusion, pointing to a genetic influence. However, the occurrence of overt diabetes, elevated fasting blood glucose or impaired glucose tolerance are also governed by external influences.

Alcohol and glucose metabolism

The relation between glucose metabolism and alcohol was reviewed by Wallgren & Barry III (1970). Blood glucose levels rise after the ingestion of alcohol, according to the amount of alcohol ingested and on the magnitude of glycogen reserves. The release of glucose from hepatic glycogen reserves into the blood is governed at least in part by the adrenal medullosympathetic nervous system (Perman 1962, Brohult *et al* 1970) since the response is inhibited by cutting or blocking of the splanchnic nerves (Perman 1962). Peripheral utilization of glucose also diminishes after alcohol ingestion (Hed & Nygren 1968).

Wallgren & Barry III (1970) were unable to find any consistent abnormalities in the glucose metabolism of alcoholics. On the other hand,

TABLE 33. Frequency of concordance and discordance with respect to fasting blood glucose > 100 mg/100 ml in relation to alcohol consumption

		LOW ALC MZ >100	MZ ≤100	MZ tot	DZ >100	DZ ≤100	DZ tot	MZ+DZ >100	MZ+DZ ≤100	MZ+DZ tot
HIGH ALC	>100	0	3	3	0	8**	8	0	11**	11
	≤100	1	10	11	0	48	48	1	58	59
	tot	1	13	14	0	56	56	1	69	70

$** = p < 0.01$

TABLE 3 I alc a Lusen re al bal c mption

Class	Discordance in alc/year (gr ms)	MZ High alc M(SD)	MZ Low alc M(SD)	No. of pairs	DZ High alc M(SD)	DZ Low alc M(SD)	No. of pairs	MZ+DZ High alc M(SD)	MZ+DZ Low alc M(SD)	No. of pairs
A	1 0t	1 10(0 51)	1 11(0 32)	5	1 13(0 36)	1 6(0 34)	9	1 13(0.38)	1.21(0.34)	30
	R r	1 85—0 70	1 43—0 77		2 79—0.70	1 99—0 68		2.29—0 70	1 98—0 68	
B	100 1 300	1 1 (0 50)	1 10(0.35)	5	1 31(0 60)	1 52(0 63)	18	1.27(0.55)	1 50(0 57)	23
	Range	1 19—0 5	1 81—1 02		2 92—0.20	3 73—0 81		2.92—0.20	3.73—0.81	
C	< 000	2 30(2 02)	1 68(0 76)	4	1 13(0.40)	1 33(0.37)	13	1 40(1 07)	1 4 (0.48)	17
	Range	5 32—1 01	2 60—0 99		1 74—0.32	2 39—0 99		5 32—0 32	2.60—0 99	
	Total	1 15(1 17)	1 38(0 51)	14	1 19(0 46)	1 36(0.47)	56	1.21(0 67)	1 36(0.47)	70
	Range	5 32—0 70	2.60—0 77		2 92—0 20	3 73—0 68		5 32—0 70	3 73—0 68	

$p < 0.05$

according to Hed (1958 a, b) and Lundquist (1965) alcoholics have a higher than normal prevalence of abnormal glucose tolerance especially in conjunction with liver derangement and increasing age. Hed (1958 a) found diabetes mellitus or fasting blood sugar > 130 mg/100 ml in 26.5 % of 67 males with portal cirrhosis of the liver mainly induced by alcohol. Oral glucose tolerance tests in 24 chronic alcoholics 7—10 days after admission to hospital (Hed 1958 b) revealed diabetogenic disturbances in 12 Ten of these subjects had cirrhosis and 2 had pronounced fatty degeneration of the liver Lundquist (1965) studied intravenous glucose tolerance (IVG) in 102 alcoholics 1—3 weeks after a drinking bout and in 27 alcoholics in a non-acute phase (hospitalized for not less than one month) Forty-one per cent of the 102 alcoholics had a k value less than 0.90, the incidence increasing with advancing age. None of the 27 patients in a post alcoholic phase had a pathological k value. Lundquist concluded that both the abnormal glucose tolerance and the pathological liver function tests could be normalized during hospital stay However the reversibility of impaired glucose tolerance in alcoholics during a hospital stay as also observed by Phillips & Safrit (1971) suggests that severe irreversible liver damage as cirrhosis is not a prerequisite for disturbed carbohydrate metabolism. A number of authors have demonstrated (Dornhorst & Ouyang 1971 Phillips 1971 Shanley *et al* 1972, Wapnic & Jones 1972 Dundee 1972) that the administration of alcohol also impairs glucose tolerance in normal subjects.

MATERIAL AND METHODS

The subject group appears in chapter I The methods applied are given in chapter II.

RESULTS

Fasting blood glucose

A significant association was found between fasting blood glucose in the pooled zygosity group and alcohol consumption (Table 3) Thu the twin in a pair with a high alcohol consumption (HAG) also had higher average fasting blood

TABLE 35 Distribution of k-value (diabetic, borderline and normal) in relation to alcohol consumption

k-value	MZ (14 pairs)				DZ (56 pairs)				MZ+DZ (70 pairs)			
	High alc.		Low alc.		High alc.		Low alc.		High alc.		Low alc.	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Diabetic	4	29		14	11	20	7	13	15	22	9	13
Borderline	3	21	2	14	19	34	8	14	22	31	10	14
Normal	7	50	10	7[illegible]	26	46	41	73	33	47	51	3

glucose value than the twin with a low alcohol consumption (LAG) Mean values amounted to 84 and 80 mg/100 ml in the HAG and LAG respectively ($p < 0.05$) When the pooled zygosity group was separated into different discordance classes, a consistently higher mean value was found in the HAG than in the LAG This was true also for the DZ group, in which the differences were most pronounced in class C. The difference was not significant for this discordance class nor for the total DZ group when it was tested using a paired "t -test, but was probably not random, as the non-parametric McNemar s test (Siegel 1956) revealed significant differences ($p < 0.05$) The greatest difference for the MZ group was found in the highest discordance class (A) in which mean values amounted to 95 and 88 mg/100 ml the HAG and LAG respectively

When fasting blood glucose was analysed with respect to a limit set at > 100 mg/100 ml (Table 33) 11 HAG twins in the pooled zygosity group exceeded this limit as opposed to one LAG twin ($p < 0.01$) No twin pair was concordant as to blood glucose > 100 mg/100 ml in either zygosity group.

Intravenous glucose tolerance

A significantly lower k value was found for intravenous glucose tolerance in the HAG than in the LAG for the pooled zygosity group (Table 34) Mean values were 1.24 and 1 36 for the HAG and LAG respectively ($p < 0.05$) Lower k values were consistently found for the HAG than the LAG in all discordance classes in the DZ group. Results for the MZ group were contrary to expectations, due to the extreme value of 5.32 obtained for one of the high consumers in the lowest discordance class (C) High consumers displayed mean k value of 1 15 and low consumers a mean alue of 1.29 when this pair was excluded.

TABLE 36. Frequency of concordance and discordance with respect to diabetic and borderline k-value ($k \leq 1.10$) in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		≤1 10	>1 10	tot	≤1 10	>1 10	tot	≤1 10	>1 10	tot
HIGH ALC	≤1 10	1	6	7	10	20**	30	11	26**	37
	>1 10	3	4	7	5	21	26	8	25	33
	tot	4	10	14	15	41	56	19	51	70

** $p < 0.01$

X

Ischemic heart disease

Investigations performed before 1965 (e.g. Hall *et al.* 1953, Howell & Manion 1960) on alcoholics with portal cirrhosis found at autopsy have revealed the presence of a thin vascular intima, particularly in the coronary arteries. After 1965 when non-cirrhotics were also included, an increasing number of reports began to be published on an association between excessive alcohol consumption and IHD (e.g. Sundby 1967, Pell & D'Alonzo 1973). As previously mentioned, the development of IHD is dependent on a genetic component. The purpose of this chapter is to describe the possible cardiovascular effects produced by longstanding use of alcoholic beverages when inter-individual variations in genetic and early environmental factors may be assumed to be minimal. A number of cardiovascular diagnoses, symptoms and findings associated with IHD were the variables examined. The subject group and methods used are described in chapters I and II.

RESULTS

Overt IHD

None of the twins in the pooled zygosity group (MZ+DZ) had been treated for myocardial infarction, and only one reported severe chest pain lasting > 30 minutes (Table 39). He was one of the alcohol-negative twins. The prevalence of

TABLE 39. Cardiovascular diagnoses, findings and symptoms in relation to alcohol consumption.

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
Myocardial infarction	0	0	0	0	0	0
Severe chest pain > 30 min.	0	1	0	0	0	1
Angina pectoris	4	2	4	3	8	5
Chest discomfort	1	4	13	13	14	17
Palpitations	0	0	14	5	14	5
Irregular heart rate		0	14	5	16	5
Slow heart rate	0	0	5	0	5	0
Intermittent claudication, suspected or confirmed	1	1	6	0	7	1
No or weak pulse in peripheral vessels	3	1	17	14	20	15
Apical systolic murmurs	7	7	30	25	37	3[illegible]
Oedema of the ankles	0	1		1		
Cyanosis	1	0	3		4	
Xanthelasma	0	0	0	2	0	2
Arcus lipoides cornea	6	6	3	19	29	25
Diabetes mellitus	0	1	2	0	2	1

[1] Angina pectoris not included
According to subjects' report

TABLE 40 Frequency of concordance and discordance with respect to angina pectoris in relation to alcohol consumption.

		LOW ALC.											
		MZ				DZ				MZ+DZ			
		angina	chest discomfort	neg	tot	angina	chest discomfort	neg.	tot.	angina	chest discomfort	neg.	tot.
HIGH ALC.	angina	1	1		4	1	2	1	4	2	3	3	8
	chest discomfort	0	1	0	1	1	5	7	13	1	6	7	14
	neg	1	2	6	9	1	6	32	39	2	8	38	45
	total	2	4	8	14	3	13	40	56	5	17	48	70

angina pectoris (for definition see p. 19) was 9.3 % in the subject group. No obvious difference was found between the high alcohol group (HAG) and the low alcohol group (LAG) in the prevalence of this symptom. Angina pectoris occurred in 8 and 5 subjects in the HAG and LAG respectively. It was present in one or both members of 11 pairs. Six of these pairs were concordant for angina pectoris or for chest discomfort (Table 40). Thus only 5 patently angina-discordant pairs could be found. These discordant pairs were almost equally distributed between the HAG and LAG. Chest discomfort, defined as chest pain not satisfying the criteria for angina pectoris, was reported by 14 and 17 subjects in the HAG and LAG respectively, 6 pairs being concordant. The results for angina pectoris and chest discomfort in both the MZ and DZ group were approximately similar.

Subjective heart symptoms

In the pooled zygosity group, there was an increased prevalence of subjective heart symptoms (palpitations, irregular heart rate, slow heart rate) for the high consumers of alcohol (Tables 39 and 41). Statistical differences were found in the incidence of irregular heart rates ($p < 0.01$) which might be due to arrhythmias, and palpitations ($p < 0.05$). The greatest disparity between the HAG and LAG twins was in the significantly larger number of combined subjective heart symptoms reported by the former group ($p < 0.01$).

Peripheral circulation

The prevalence of clinical findings of impaired blood flow to the feet was approximately similar in the HAG and LAG in the pooled zygosity group (Tables 42 and 43). Suspected or manifest intermittent claudication was reported by 7 subjects in the HAG and 1 subject in the LAG respectively ($0.1 > p > 0.05$). Twenty twins of the HAG and 15 twins of the LAG had absent or only weak pulsations in the dorsal arteries of the feet on physical investigation. However suspected or manifest intermittent claudication was found in 6 of the HAG but in none of the LAG when the DZ group was analysed separately. This difference between the two groups is significant ($p < 0.05$). The fact that 17 HAG twins and 14 LAG twins in the DZ group had weak or absent pulsations in the dorsal pedal arteries provided no statistical support for the prevalence of suspected or confirmed intermittent claudication.

Other diagnoses, symptoms and findings associated with IHD

The prevalence of manifest diabetes mellitus in the subject group was about 2 % (Table 39) which is about the average in a large population (Marble et al. 1971). The small number of subjects with this disease made it impossible to draw any conclusions. Differences between the high and

TABLE 41 F quency f concordance and discordance with spect t subject heart sympt ms in elation to al ohol con umption

			LOW ALC								
			MZ			DZ			MZ+DZ		
			pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	Palpitations	pos	0	0	0	1	13*	14	1	13*	14
		neg	0	14	14	4	38	42	4	52	56
		tot	0	14	14	5	51	56	5	65	70
	Irregular heart rate	pos	2	2	2	2	12*	14	2	14**	16
		neg	0	12	12	3	39	42	3	51	54
		tot	0	14	14	5	51	56	5	65	70
	Slow heart rate	pos	0	0	0	0	5	5	0	5	5
		neg	0	14	14	0	51	51	0	65	65
		tot	0	14	14	0	56	56	0	70	70
	All categories of subjective heart symptoms	pos	0	2	2	2	19**	21	2	21**	23
		neg	0	12	12	6	29	35	6	41	47
		tot	0	14	14	8	48	56	8	62	70

* = $p < 0.05$
** = $p < 0.01$

low alcohol consumers as regards findings such as cyanosis, oedema of the ankles or xanthelasma were negligable. Nor was the finding of systolic murmurs or arcus lipoides cornae, the prevalence of which were high, more common in the high than in the low alcohol group.

Radiographic examination of the heart

Disparity was found in the relative heart volumes (Jonsell 1939) in the highest discordance class (A) of the pooled zygosity group (Table 44) The mean volumes amounted to 366 and 337 ml for the HAG and LAG respectively ($p < 0.05$) The difference is certainly statistically significant but not consistent with the over all pattern and is judged as having no clinical significance. The opposite trend was noted in the lower discordance classes (B and C)

Heart rate at rest

In the pooled zygosity group the HAG had a higher resting heart rate (Table 45) than the LAG ($p < 0.05$) The registered mean heart rates were 71.2 and 68 0 beats/min in the HAG and LAG respectively Rising alcohol discordance did not increase this difference in pulse rate.

ECG findings at rest

The resting ECG in the pooled zygosity group disclosed a nonsignificantly higher number of

TABLE 42 Frequency of concordance and discordance with respect to suspected or confirmed intermittent claudication in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		susp pos	neg	tot	susp pos	neg	tot	susp pos	neg	tot
HIGH ALC	susp pos	0	1	1	0	6*	6	0	7	7
	neg	1	12	13	0	50	50	1	62	63
	tot	1	13	14	0	56	56	1	69	70

* $= p < 0.05$

twins with left axis deviation (Table 46) in the high alcohol group than in the low alcohol group. This disparity was due to anterolateral bundle branch block in 3 of the 4 observed cases. One subject in the low alcohol group had Q waves not definitely diagnostic of previous infarction (Blackburn *et al* 1960) With respect to the ST segment at rest, 4 and 3 subjects were coded 4 1—3 (for definitions see p. 22) in the HAG and LAG respectively (Tables 46 and 47) The only subject in the study who had atrial fibrillation was found in the high alcohol group.

ECG during and after exercise

The number of twin pairs concordant and discordant for segmental ST depression during exercise in relation to alcohol consumption is illustrated in Table 47 No differences in ST changes at maximal comparable work load were evident between the HAG and LAG twins in the pooled zygosity group. Of the 69 twin pairs whose response to exercise could be compared, 16 subjects in the high alcohol group and 13 in the low alcohol group were classified according to ST code 1—3 Six pairs were concordant according to this criterion. The prevalence of pathological ECG findings during maximal exercise was found to be somewhat higher in the MZ than in the DZ group. A reduced prevalence of ST changes was found in both the HAG and the LAG immediately after and 3 min after work. There were no ECG disparities between the high and low alcohol group

TABLE 43 Frequency of concordance and discordance with respect to absent or weak pulsation in dorsal pedal artery in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
		pos	neg	tot	pos	neg	tot	pos	neg	tot
HIGH ALC	pos	1	2	3	3	14	17	4	16	20
	neg	0	11	11	11	28	39	11	39	50
	tot	1	13	14	14	42	56	15	55	70

TABLE 44 Relative heart volume in ml/m² BSA in relation to consumption

Class	Discordance in abs. alc./year (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No. of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No. of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No. of pairs
A	> 10,000	350(59)	361(68)	5	370(81)	332(47)	25	366(77)	337(49)	30
B	5,000—10,000	328(13)	384(71)	5	346(53)	365(61)	18	342(47)	370(65)	23
C	< 5,000	348(79)	363(67)	4	337(54)	352(54)	12	339(58)	355(55)	16
	Total	341(52)	370(64)	14	355(67)	348(55)	55	352(64)	352(57)	69

= $p < 0.05$

Arrhythmias

No obvious differences in the prevalence of arrhythmias at rest were found between the HAG and LAG in the pooled zygosity group (Table 46). There was an increased incidence of arrhythmias in both alcohol groups during and after exercise (Table 48). A total of 13 subjects, all belonging to the DZ group, had arrhythmias classified into codes 1—6 in accordance with Table 48. No obvious disparities were found between the high and low alcohol consumers.

Comments

The most serious arrhythmia that occurred was a brief episode of ventricular tachycardia. This arrhythmia was seen in an alcohol-positive twin of the DZ group shortly after exercise and during continuous ECG registration. The arrhythmia spontaneously reverted to sinus rhythm, but the subject was moved immediately to the coronary care unit. Since the serum enzyme values were not elevated, no new episodes of ventricular tachyarrhythmias occurred during continous monitoring and no ECG changes suggestive of myocardial infarction appeared, he was permitted to leave hospital 24 h later.

DISCUSSION

The main finding in the present study was the absence of "hard IHD", i.e. verified myocardial infarction or death due to IHD (Keys *et al.* 1972 b). Myocardial infarction is uncommon in young or middle-aged Swedish men (Biörck *et al.* 1966, Tibblin 1967, Bengtsson 1973). Tibblin (1967) found a 1 % prevalence of myocardial infarction in Swedish men aged 50. In Stamler's review (1973) Sweden was one of the industrialized countries with the lowest incidence of IHD death in the 25—64 age group. In Swedish males "hard IHD" (Keys *et al.* 1972 b) occurs com

TABLE 45 Heart rate at rest in relation to alcohol consumption (beats/min)

Class	Discordance in abs. alc./year (grams)	MZ High alc. M(SD)	MZ Low alc. M(SD)	MZ No. of pairs	DZ High alc. M(SD)	DZ Low alc. M(SD)	DZ No. of pairs	MZ+DZ High alc. M(SD)	MZ+DZ Low alc. M(SD)	MZ+DZ No. of pairs
A	> 10 000	74.2(1.5)	75.6(6.5)	5	70.4(14.7)	66.6(13.0)	25	71.0(13.5)	68.1(12.5)	30
B	5,000—10,000	68.6(9.2)	65.2(14.3)	5	69.6(15.5)	68.5(16.0)	18	69.4(14.2)	67.7(17.2)	23
C	< 5,000	67.3(5.7)	71.5(13.6)	4	76.1(10.3)	67.2(11.5)	15	74.0(10.1)	68.2(11.8)	17
	Total	70.2(6.7)	70.7(11.8)	14	71.5(14.1)	67.4(13.5)	56	71.2(12.9)	68.0(15.1)	70*

= $p < 0.05$

TABLE 46. ECG changes at rest in relation to alcohol consumption.

	MZ (N=14 pairs)		DZ (N=56 pairs)		MZ+DZ (N=70 pairs)	
	High alc.	Low alc.	High alc.	Low alc.	High alc.	Low alc.
Q wave pathological	—	—	—	1	—	1
Axis deviation, left	—	—	4	1	4	1
ST segment						
ST Code 1	—	—	1	—	1	—
2	—	—	1	—	1	—
3	—	—	2	3	2	3
4	—	1	1	—	1	1
5	5	4	8	5	13	9
6	—	—	—	—	—	—
7	—	—	1	—	1	—
T-wave						
> —3 mm	—	—	—	—	—	—
—1 to —3 mm	—	—	1	—	1	—
Flat or diphasic	—	—	1	—	1	—
Low amplitude	1	—	2	5	3	5
Anterolateral bundle branch block	—	—	3	1	3	1
Sinus tachycardia	—	—	2	2	2	2
Arrhythmias:						
Atrial fibrillation	—	—	1	—	1	—
Frequent multifocal VEB	—	—	—	—	—	—
Frequent unifocal VEB	—	—	—	—	—	—
Occasional multifocal VEB	—	—	—	—	—	—
Occasional unifocal VEB (2—4 in 20 complexes)	—	—	—	—	—	—
Occasional VEB	1	—	2	1	3	1
Frequent SVEB (> 5 in 20)	—	—	—	1	—	1
SVEB (2—4 in 20 complexes)	—	—	—	—	—	—
Occasional SVEB	—	—	—	—	—	—

monly at an advanced age. Since none of the investigated twins had suffered from a myocardial infarction, the 10 deceased twins in the subject group were also traced (p. 15). The death of three in the high alcohol group and one in the low alcohol group proved to be associated with IHD.

Thirteen persons (9.3 %) in the entire subject group were angina pectoris-positive, with a slightly higher prevalence found in the high (11.5 %) alcohol group than in the low (7.1 %) alcohol group. The number of positive answers was somewhat higher than the number usually reported. In his study of smoking-discordant twins Lundman (1966) found angina or suspected angina in 7 % of his subjects. However, the average age was about 50 in his investigation as compared to 55 years in the group presented here. Rose (1968) found that the prevalence of angina pectoris was relatively constant at approximately 4 % by repeated assessment over a 4-year period of a sample of 1,136 men initially aged 35—59. The large majority were positive on only a single occasion and the 4-year period prevalence was 10.4 %.

It was of interest to note in the present study that angina pectoris was not less prevalent in the high than in the low alcohol group. Earlier

TABLE 47 Frequency of oncordance and discordanc th pect to ST egment d pression at rest and during maximal omparabl ork in relation to al ohol on mption

			LOW ALC								
			MZ			DZ			MZ+DZ		
		ST-code	1-3	0 or 4-7	tot	1-3	0 or 4-7	tot	1-3	0 or 4-7	tot
HIGH ALC	At rest	1-3	0	0	0	1	3	4	1	3	4
		0 or 4-7	0	14	14	2	50	52	2	64	66
		tot	0	14	14	3	53	56	3	67	70
	Max comp work	1-3	2	2	4	4	8	12	6	10	16
		0 or 4-7	1	9	10	6	37	43	7	46	53
		tot	3	11	14	10	45	55	13	56	69

questionnaire-studies of the Swedish and U.S. twin registries have shown that the positive members of pairs discordant with respect to alcohol consumption had significantly increased rates of angina pectoris among both DZ and MZ twins (Twin Registries in the study of chronic diseases 1971) Alexander (1966) suggested "that angina is conspicuously absent in alcoholics. This may well be true of alcoholics but not of subjects whose consumption of alcohol is only moderately excessive.

Many authors have stressed that angina pectoris is a fairly unreliable symptom of IHD (e.g. Lundman 1966, Lundman *et al* 1971 Raftery *et al* 1971 Keys *et al* 1972 b) Rose (1961) has shown that respiratory symptoms in smokers may result in a false positive diagnosis of angina pectoris. This might influence assessments of the prevalence of angina to some extent, especially among high alcohol consumers who were found to be heavy consumers of tobacco also (chapter IV)

The present interview results showed that subjective heart symptoms (palpitations, irregular heart rhythm and slow heart rate) occurred more frequently among high than among low alcohol consumers. These results were not verified by ECG during and after exercise. However the subjective symptoms reported above should not be set aside as the subjective reports refer to many years of observation as compared to a single ECG record Earlier studies have disclosed link between high alcohol intake and cardiac arrhythmias (Fredriksen & Hed 1958, Jörgensen & Hedebo 1968, Carlsson 1970) Vetter *et al* (1967) found a correlation between the presence of cardiac arrhythmias and subnormal serum potassium in acute alcohol withdrawal. A low serum potassium level in alcoholics was also reported by Johnsson and a direct toxic action by alcohol on the heart symptoms in the alcohol positive group could be increased secretion of adrenaline after ingestion of alcohol (Perman 1962, Brohult *et al* 1970) and a direct toxic action by alcohol on the heart (Alexander 1966, Brandfonbrener 1967) James & Bear (1967) showed that acetaldehyde which is a metabolite of alcohol, had a direct effect on the sinus node, mainly due to its noradrenaline-releasing effects.

The results point to a link between alcohol consumption and resting heart rate. In any case, this variable did not discriminate strongly between the high and low alcohol group in the present study

TABLE 48 Frequency of concordance and discordance with respect to total duration of arrhythmias during and after exercise in relation to alcohol consumption

		LOW ALC								
		MZ			DZ			MZ+DZ		
	code	1–6	0 or 7–10	tot	1–6	0 or 7–10	tot	1–6	0 or 7–10	tot
HIGH ALC	1–6	0	0	0	1	4	5	1	4	5
	0 or 7–10	0	14	14	7	43	50	7	57	64
	tot	0	14	14	8	47	55	8	61	69

Code
- 0 No arrhythmia
- 1 Ventricular tachycardia
- 2 Atrial fibrillation
- 3 Frequent (more than 5 out of 20) multifocal ventricular premature beats
- 4 Frequent (more than 5 out of 20) unifocal ventricular premature beats
- 5 Multifocal (2 to 4 out of 20) ventricular premature beats
- 6 Unifocal (2 to 4 out of 20) ventricular premature beats
- 7 Occasional ventricular premature beats
- 8 Frequent (more than 5 out of 20) supraventricular (including nodal) premature beats
- 9 Supraventricular (including nodal) premature beats (2 to 4 out of 20)
- 10 Occasional supraventricular (including nodal) premature beats

In the case of more than one type of arrhythmia, each subject was only coded once in accordance with the lowest code number

Mild tachycardia is usually encountered in studies of alcoholics (Fredriksen & Hed 1958, Evans 1959 Brigden & Robinson 1964, Carlsson 1970 German 1973) Insurance data have suggested a relationship between casual determinations of resting heart rate and subsequent mortality (Stamler 1973) Berkson *et al* (1970) reported that a heart rate of > 80 beats per minute was associated with higher 10-year IHD mortality rates. On the other hand, Blackburn *et al* (1970) was unable to find any excess 5 year IHD incidence in initially IHD-free subjects with resting heart rates of more than 100 beats/minute.

Radiographically determined heart volume displayed significant disparity in the highest discordance class of the DZ group. This result was not confirmed in any other subgroup and is not considered to be of clinical importance. It is surprising that the link between alcohol consumption and relative heart size was not more pronounced. Even though most of the twins in the high alcohol group were moderate alcohol consumers, a few subjects had a consumption adequate for the development of cardiomyopathy (Evans 1957 Brigden & Robinson 1964, Brandfonbrener 1967) Hypertension may be another possible reason for a relatively enlarged heart volume (Humerfelt 1963 Tibblin 1967) This condition was found to be more prevalent in the alcohol-positive group (chapter V)

Electrocardiography during and after exercise is regarded as a fairly reliable method for the detection of subclinical IHD (Blomqvist 1965 1971 Åstrand 1973 Helfant *et al* 1973) This method also increases the possibility of a proper evaluation of arrhythmias (Kosowsky *et al* 1971) Since it has been shown (Blomqvist 1965) that the magnitude and frequency of ST segment depression in

exercise is directly related to the relative workload, it is important to keep this sufficiently heavy. The subjects in the present study were asked to continue exercising "as long as possible," and so high workloads were obtained (average for the entire subject group 140 Watts). Thus, few affected subjects are assumed to have passed through the investigation without IHD being detected.

The workload for each subject of a pair must be about equal if the intra pair comparison is to be valid. Thus the ECG records made during exercise were interpreted at the highest comparable heart rate, i.e. the highest heart rate recorded by the twin with the lowest final heart rate of a pair.

Follow-up studies of asymptomatic subjects have demonstrated that a horizontal ST depression, especially during or after exercise (Mattingly 1962, Blomqvist 1965, Mason *et al.* 1967, Åstrand 1973), is associated with a high risk of the development of clinical IHD. In a 10-year follow-up study of about 1 000 subjects, Mattingly (1962) showed that the incidence of IHD was about 8 times higher after 10 years in subjects with ischemic ST depression after exercise than in subjects without this. Mason *et al.* (1967) found a 81 % sensitivity for positive ST changes in comparison with significant changes in coronary arteriography. According to Blomqvist (1971) the prognostic significance of a pathologic exercise ECG appears to be independent of other known risk factors. ECG studies of alcoholics have shown that the most commonly encountered findings are mild tachycardia, extrasystoles, atrial fibrillation and conduction disturbances (Fredriksen & Hed 1958, Evans 1959, Brigden & Robinson 1964, Carlsson 1970, German 1973). Evans (1959) identified T wave deformation of the ECG as a distinctive feature. Levine *et al.* (1965) and Priest *et al.* (1966) demonstrated that the deformed T wave is a good diagnostic criterion of alcoholic cardiomyopathy. Since practically none of the alcohol-positive twins showed T wave changes, it seems reasonable to assume that few of the subjects had any cardiomyopathy. This statement is also supported by the X-ray findings indicating that relative heart volumes were essentially the same for the high and low alcohol consumers. In addition, the clinical examination provided no basis for the diagnosis of cardiomyopathy.

No obvious differences were found between the high and low alcohol consumers with respect to IHD, judged on the basis of clinical or ECG findings at rest and during exercise. Thus, these results do not point to an association between long lasting moderate consumption of alcohol and IHD or cardiomyopathy in males when the influence of genetic and early environmental factors was assumed to be minimal. The results could possibly be ascribed to the fact that the examined twins were too young for detection of IHD using the methods applied, and to the influence of genetic factors.

SUMMARY

Seventy alcohol-discordant twin pairs were investigated with respect to symptoms, signs and diagnoses associated with IHD. None of the twins had suffered from myocardial infarction, and the prevalence of angina pectoris was essentially the same in the high and low alcohol groups. There is no evidence indicating that any of the subjects had cardiomyopathy. In the pooled zygosity group (MZ+DZ) reported subjective heart symptoms, such as palpitations and sensations resembling arrhythmias, appeared more frequently in the high rather than in the low alcohol consumers. A weak significance was also found for this group as regards elevated heart rate and as regards a slight increase in relative heart volume in the highest discordance class. The ECG at rest and during exercise showed no significant difference between the high and low alcohol consumers. It may be concluded that there is no apparent association between alcohol consumption and IHD when the influence of genetic and early environmental factors is minimal.

XI

General discussion

Studies of the possible somatic effects of alcohol consumption have usually employed subjects who were obvious alcoholics. IHD investigations prior to 1965 dealt mainly with alcoholics with cirrhosis of the liver, a disorder seldom associated with atherosclerosis, but with an unfavourable prognosis. Epidemiological studies have shown that subjects with an excessive consumption of alcohol are more prone to IHD than the rest of the population. Furthermore, non-vagrant alcoholics have been reported to be more prone to IHD than vagrant subjects (Sundby 1967). It is evident that moderately excessive alcohol consumers, gainfully employed and apparently well adapted socially have seldom been included in earlier studies. These subjects represent the vast majority of alcohol consumers. It was thus considered of major interest to investigate the possible effect of this habit on the appearance of factors associated with IHD subclinical as well as manifest disease.

It is well known that subject's own account of his alcohol consumption is often biased due to his inclination to reply in accordance with social desirability. In the present study the classification of alcohol consumption is considered reliable. On reassessing each twin's consumption 1972/73 as compared to 1967 it was found constant relative to his co-twin. Repeatability was thus 100 %. The following criteria of validity were studied in detail: specific anamnestic variables, physical findings and serum enzymes associated with a high alcohol intake. These items disclosed significant disparities between high and low alcohol consumers, with the exception of findings indicative of more serious somatic damage, such as jaundice, ascites and pathological serum enzymes. The results, including these exceptions, confirmed the presence of moderate alcohol consumption in the high alcohol group and that the classification of alcohol ingestion must be considered valid.

The role of hereditary factors in various diseases should be taken into consideration more often than has been the case, especially when studying the etiologic importance of an environmental factor in the development of disease. Since hereditary factors have been *found to play an important role* in both drinking habits and in the development of IHD it was considered important to minimize their influence, as far as possible, by investigating group of alcohol-discordant twins. The twin method means that the influence of genetic factors is minimized and that differences in early environment are also to a great extent reduced. In addition, perfect age matching is obtained.

Using monozygotic twin pairs, the influence of genetic factors is completely eliminated. Furthermore, genetic factors are shared to about 50 % in a pair of same-sexed dizygotic twins. In the present study results were mainly derived from the dizygotic series. In the pooled zygosity group (MZ+DZ) however the influence of genetic factors was eliminated to 60 % due to the fact that the study group comprised 14 MZ and 56 DZ twin pairs. The present study satisfactorily reduced the influence of genetic factors in comparison with conventional studies of probands and control subjects.

Thirteen of the initial 92 pairs were lost due to death (8 pairs) or severe illness. The cause of death in relation to previously reported alcohol consumption was ascertained. The number of pairs investigated (70) deviated only slightly from the target group (79 pairs). This must be considered satisfactory as many of the subjects had to travel more than 500 miles in order to reach the examiner. Nine pairs refused to participate or were unable to take part but all were contacted by telephone. The loss of a pair was often due to one of its members being unable to make the journey because of his intemperate habits. So these losses

most probably reduced the possibility of showing differences between the groups.

Even though the Swedish Twin Registry contains a large material, only 86 patently alcohol-discordant twin pairs could be found. This was because of the criteria for selection, age, sex and alcohol discordance. Moreover the twins showed a high rate of concordance with respect to intra pair drinking habits. A carefully devised clinical study was still considered of value in showing whether these alcohol-discordant twin pairs differed regarding factors associated with IHD subclinical as well as manifest disease. In evaluating the results of the present study the difficulties arising from the relatively limited number of subjects making especially the negative statements inconclusive, must be conceded. The difference between stating that "an effect is absent and that "an effect can not be demonstrated is obvious.

It is evident from the present study that moderate, longstanding alcohol consumption is associated with other factors known to be related to the development of IHD An obvious link was recorded as regards smoking. It is well known that alcohol consumption is associated with this habit. So the problem must be faced whether the results in the present study can be explained by smoking alone. That this is not so is evident from the following table:

It is apparent that factors known to be associated with the development of IHD are present at higher levels in the high alcohol group than in the low alcohol group even when the effect of smoking is eliminated. In a study of smoking-discordant twin pairs, Lundman (1966) was able to disclose an inverse relationship between smoking and blood pressure, weight, serum cholesterol and serum triglycerides. Using a multiple logistic model on a random sample, Wilhelmsen *et al* (1973) demonstrated the absence of any correlation between smoking and blood pressure or serum lipids In an epidemiological study of asymptomatic hyperuricemics, Fessel *et al* (1973) was unable to show that cigarette smoking had any significant effect on serum uric acid. The same has also been suggested with respect to intravenous glucose tolerance (Wahlberg 1966) So, the metabolic effects in humans of even moderate doses of alcohol are considerable and may be compared to an extreme exposure to tobacco. Except for the induction of symptoms such as cough, phlegm, asthma, and dyspnea, the higher daily cigarette consumption in the high alcohol group can not explain the increased prevalence of factors associated with IHD

It is well known that many of the metabolic variables, e.g. fasting blood glucose and serum uric acid, are related to other variables, such as

Examined variables in relation to alcohol consumption in 11 twin pairs concordant with respect to non smoking

	MZ+DZ (N=11 pairs)				
	High alc		Low alc		
	M(SD)				
Casual systolic blood pressure	151	(15)	147	(19)	mm Hg
Basal systolic blood pressure	93	(11)	89	(9)	mm Hg
Absolute weight	72	(12)	68	(9)	kg
Serum cholesterol	242	(37)	232	(44)	mg/100 ml
Serum triglycerides	129	(45)	148	(72)	mg/100 ml
Serum uric acid	5,8	(11)	5,2	(8)	mg/100 ml
Fasting blood glucose	83	(21)	81	(10)	mg/100 ml
IVGT (k value)	5		2		

Number of subjects with borderline or pathological values

serum cholesterol and blood pressure, in a complicated metabolic network (Epstein 1973 a) The present investigation suggests that long-standing moderate alcohol consumption has a number of physiological and metabolic effects. Thus, systolic and diastolic blood pressures, fasting blood glucose and serum uric acid were significantly higher in the high alcohol group than in the low Significant differences were also found for intravenous glucose tolerance and the intra-pair level of serum cholesterol, the high consumers showing the higher number of pathological or elevated values That elevated and even pathologically high levels are confined to the high alcohol group is striking.

As the present investigation was performed after at least 12 h of fasting no alcohol was consumed by the twins from the evening before That this was so in all cases but one was verified by means of a blood alcohol assay Casual measurements of metabolic variables such as blood glucose serum lipids, serum uric acid etc., have been performed in some large population studies Results from such investigations might partly be explained by the acute effects of alcohol. Nevertheless, such casual measurements are of great value, as they most probably give a truer picture of the subject s normal condition

Sensitive methods are important when conclusions are to be drawn regarding IHD In the present study the resting ECG was supplemented by an exercise ECG a method considered fairly reliable in the detection of IHD (e g Blomqvist 1971 Astrand 1973) The value of the ECG during exercise was further enhanced as all subjects but one were able to carry out the test, in most cases at a submaximal level.

None of the twins had suffered from myocardial infarction The prevalence of angina pectoris was higher but not significantly so in the high alcohol group than in the low The ECG at rest or during exercise did not differ between the twin groups. Thus, no differences could be demonstrated between the high and low alcohol consumers with regard to manifest or subclinical IHD This may be explained by the small size of the sample by genetic factors, and by the methods available for the detection of IHD being comparatively crude for intra-pair comparison. Another possible explanation is the low mean age of the sample the twins examined might still be too young to have developed IHD Therefore a follow p of the present subject group will be performed.

General summary and conclusions

The main purpose of this study was to investigate if there is a relationship between alcohol consumption and factors associated with IHD subclinical as well as overt disease, in apparently healthy gainfully employed men when the influence of genetic and early environmental factors was minimal.

Anamnestic variables, somatic findings and some serum enzymes associated with a high alcohol intake were also studied, mainly in order to establish that the selected twin sample really consisted of moderately alcohol-discordant twin pairs. Significant differences were obtained between the twins for the following anamnestic variables: blackouts, eyeopeners, intoxication, heavy consumption at parties, "more than half a bottle on the same occasion" and subjective classification of personal alcohol habits. In addition, the alcohol positive group showed a significantly increased prevalence of hepatomegaly, tremor, vascular spiders—"paper money" skin and Dupuytren's contracture but not of more serious findings, such as jaundice and ascites. Serum enzymes also showed significant disparities for SGOT, SGT and SALP, the slightly higher but almost consistently nonpathological values being found among the high alcohol consumers. On the basis of these results and the calculations of alcohol consumed per year the twins were designated moderately alcohol discordant and assigned to a high alcohol group (HAG) and a corresponding low alcohol group (LAG).

The material comprised 70 pairs, 14 monozygotic and 56 dizygotic same sexed male twin pairs, aged 45—65 years. They made up 76 % of a sample selected from the Swedish Twin Registry containing some 4 500 male pairs, compiled at the Department of Environmental Hygiene, Karolinska Institute, and the National Institute of Public Health, Stockholm. The chief selection criterion was discordance with respect to alcohol consumption set at > 10 000 and < 2 000 g of absolute alcohol/year.

A sociological and medical history was taken using a standardized interview. The cardiovascular questions were designed by the London School of Hygiene and Tropical Medicine. The zygosity classification was obtained by serological determinations carried out at the National Laboratory of Forensic Medicine in Stockholm.

The subjects were submitted to an examination, including anthropometric and blood pressure measurements, radiographic examination of the chest and an electrocardiographic examination before, during and after a maximal exercise test. Blood samples were drawn after 12 hours of fasting and analyzed for serum cholesterol, serum triglycerides, blood glucose, serum uric acid and alcohol in blood. In addition an intravenous glucose tolerance test and lipoprotein electrophoresis of serum were performed.

The hypotheses stated in the present study (page 12) were confirmed in the pooled zygosity group (MZ+DZ) in respect to the following findings:

1. Smoking was found slightly more often in the high alcohol group. Pipe and cigar smoking was significantly more frequent among the low consumers of alcohol. Quantitative cigarette consumption showed the most striking differences, and subjects in the high alcohol group smoked significantly more cigarettes daily. These differences applied to present smokers but were also applicable when former smokers were included. Significant disparities in the prevalence of pulmonary symptoms, such as cough, phlegm, dyspnea, and asthma, were found: all these symptoms were more common in the high alcohol group.

2. A greater number of the high alcohol consumers than the low had previous history of hypertension. A significant link between alcohol consumption and current blood pressure level was also obtained. This was true of both systolic and diastolic pressures. The findings were independent of whether measurement was casual or basal.

3. No significant association was found between alcohol consumption and any weight parameter. The alcohol-positive twins in the highest discordance class had a significantly thicker layer of subcutaneous fat in the triceps area than their low consumption co-twin. No disparities were found for the subscapular area.

4. Small and almost consistently non-significant differences were found in serum lipids. No evident disparities were observed in mean values for serum cholesterol, triglyceride levels, and serum lipoproteins. A significantly greater proportion of the high alcohol consumers exceeded the > 200 mg/100 ml limit for serum cholesterol in the intrapair evaluation.

5. A significant association was found between alcohol consumption and fasting blood glucose, and significantly more of the high consumers had fasting blood glucose values > 100 mg/100 ml. Significantly lower k values were noted for intravenous glucose tolerance among high consumers. The prevalence of "borderline" or diabetic k-values was 53 % in high alcohol consumers and 27 % in low alcohol consumers.

6. Significantly higher levels of serum uric acid were found in the high alcohol group. The greatest difference was obtained in the highest discordance class. Serum uric acid ≥ 6 mg/100 ml was also significantly more common in the high alcohol group.

7. There was no excess morbidity owing to overt IHD or cardiomyopathy among the high alcohol consumers. None of the twins had suffered from myocardial infarction, and the prevalence of angina pectoris was higher but not significantly so, in the high than the low alcohol group. The prevalence of subclinical IHD or arrhythmias, as shown by the ECG at rest or during exercise was the same for both groups. A significantly greater number of the twins in the high alcohol group than in the low reported subjective heart symptoms, such as palpitations and sensations which could be described as arrhythmias.

CONCLUSIONS

On the basis of the present study significant differences were established for moderately alcohol-discordant male twin pairs, aged 45—65 years, for systolic and diastolic blood pressures, high cigarette consumption, phlegm, cough, asthma, dyspnea, serum cholesterol values > 200 mg/100 ml, hyperuricemia, elevated fasting blood glucose and diabetic k values; the greater number of findings or pathological values were found in the high alcohol consumption compared to the low alcohol consumption co-twins. No conclusion can be drawn with respect to overt or subclinical IHD while statistically significant disparities were found with regard to subjective heart symptoms, such as palpitations and sensations possibly indicative of arrhythmias.

Despite obvious differences in the prevalence of number of conventional risk factors, no disparity has yet been found in that of manifest IHD between subjects with high or low alcohol consumption. This finding may well be due to the limited size of the subject group, to the low prevalence of IHD anticipated in a group with such a low mean age, or to its constitutional similarity

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Appendix

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Ischaemic Heart Disease in Death Discordant Twins

A Study on 205 Male and Female Pairs

By Ulf de Faire

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Ischaemic Heart Disease in Death Discordant Twins

A Study on 205 Male and Female Pairs

By Ulf de Faire

STOCKHOLM 1974

CONTENTS

An in rea ing mortality from i chaemio h art di eas (IHD) during the la t d cad e p cially in m n ha b n report d by v ral authors (d Haa 1964; Oliver & Stuart Harri 1965; R ader & Wynn 1966; Stamler 1973) However figure from mortality tati ti in Sweden show only a slight in r a e in m n and a corr p nding d - r a e in women (Biörok & Bylin 1965; Biörck et al 1970; V din et al 1971) In o mparis ns between countri mal IHD mortality i high t in Finland and th USA while Swed n ome omparatively low down (Blomqvist & Biörck 1963; Moriyama et al 1971)

IHD i no doubt a major international problem cau ing early d ath and invalidity Th Nati nal H alth Examination Surv y ha hown that veral million p r on in the USA hav IHD definite or susp ct (Ath ro cl rosi Study Gr up 1970) Thu it i und r tand- able that gr at eff rt ar n w c n ntrat d on trying to prevent or at l a t d lay th development of IHD (primary prev ntion) as well a trying to prevent relap (se ondary prev ntion) Howev r prev ntive acti n presuppo knowl dge of the pertin nt etiologi al factors

Pro p tiv p pulation tudi hav demonstrated a ciations b tw n the in id n e of IHD and differ nt variabl usually called risk fa t r By ri k fa tor ar und r tood th e fa t r which are tatistically as o iated with IHD but thi do not n arily imply a cau al relationship

Many ext n iv revi w c nc rning ri k fa tors hav b n pub- li hed (Kannel t al 1961; Biörck 1963; K y & Blackburn 1963; Ep tein 1967; Simb rg 1970; F jfar 1972; Staml r 1973) Judging from ev ral pro p tiv tudi th major c ntributory fa tor em t be hypert nsi n hyperchol terolaemia and smoking (Ath ro lero i Study Group 1970) Th ir impact n risk i more pronounc d wh n th y ar pre nt in ombination Other risk factor hav also be n id ntified a for xampl hypertriglyceridaemia variou hyper lip proteina mia overweight edentary living psycho- ocial t nsions di tary fact rs x essiv alcohol con umption hyper- uricaemia and diabet mellitus Th risk factor can be subdivided int biometri r intrinsi and environmental or extrinsic The ro t of many biometric fa tor ar probably mo tly g n ti but th y may be su ptibl t th influ nc of nvironment Similarly

environmental factors may be linked in some measure to the genetic constitution (Biörck 1959)

The importance of the hereditary influence in IHD has always b n stress d by Paul D Whit (1957 and 1960) The general approache to determining the strength and mechanism of genetic factors in chronic diseases have b n describ d among others by Penrose (1953) and Edwards (1963) The meth ds available for ob ervational study of th genetic basi of IHD have usually been the study of familial aggregation and twin tudies

Familial aggregation of i chaemic h art disease

Familial aggregation of IHD has often b en accepted a an indication of a sub tantial genetic influence on IHD Several studie have demonstrated such aggregation (Gertler & White 1954; Thomas & Cohen 1955; Ru ek & Zohmann 1958; Shanoff et al 1961; Rose 1964) H w ev r a pointed out by Mc Kusick and Murphy (1963) mode t familial concentrations have b en found for most common disea es for which they have be n ought A contributory fa tor to th familial cluster ing of IHD found in retro pective tudie could be that probands are more aware of relatives with the trait in que tion In a report from the Tecumseh Community H alth Study Napier et al (1972) hav shown that family history report on morbidity are rather limit d Th finding also suggest d that mortality data obtained exclusively from family historie should be interpreted with great caution

Prospective tudies hav also di clos d familial aggr gation of IHD (Slack & Evans 1966; Deut cher et al 1970; Hammond et al 1971) But according to Epstein (1964) the relative importance of familial influences in the genesi of IHD annot be e timated quantitatively from th findings of family studi It must be born in mind that the members of a family tend to shar not only their g ne but th ir environment

Twin studies

Ever since the day of Galton (1822-1911) twins have be n tudied with regard to th gen tic influen e on chronic disea e The p s ible contribution of twins in epidemiologic studi s of chronic di as wa revi w d at a m eting in 1965 sp nsor d by WHO It wa str s ed that twin tudies offer a u eful tool in evaluating the relative importan e of heredity and nvironment for chroni condi- tions su h as IHD The methodology in twin tudi has b en carefully

analysed by C d rlöf (1966) and oth r in luding an international ymposium in Pu rto Rico in 1969 Sev ral models have b n sugge ted for testing specific hyp these The most common t chnique i probably the called cla sical twin method de ribed by everal author (Dahlb rg 1926; von V rschu r 1958; Gedda 1961; Harvald & Hauge 1965) Here the rate of concordanc for one p ific trait i ompar d b tween monozygoti (MZ) and dizygotic (DZ) twin pairs A ignificantly high r d gree f con ordanc in the MZ pair speaks in favour of geneti factors b ing r ponsible for th diff r nce Thi con lusion a ume h wev r that th intra pair differen e for MZ and DZ twins with re pect to environmental fa tors i of ab ut th am ord r which may n t be the cas Another a sumption i an unbias d selection of pair but in pra tic there i often an over r pr entation of pairs with th trait und r inve tigation

A imilar approach used by Kaij (1960) and Ced rlöf et al (1970) i to compare th b rved and xpected rate of oin id n e The ob erved rat i d fin d a th ratio between th numb r of pairs where both twins hav th trait and th total number of pair b rved Th xpected rate i calculated from th prevalen in the twin sample Th gen tic dependence of c ntinuous variable and th influ n on them of environment are be t studied by varian e analysi (O born & d George 1959; Takkunen 1964) A le marked intra pair differ n in MZ pairs compared to DZ pairs indicates that the variabl in qu tion i more dep nd nt on g n tic than on nvironmental factor

Th co twin control method worked out by G ll (1942) i e p ially suitable for tudying th influen of sp cifi environmental fa tors n h alth. It has th advantage of keeping the genetic fa t r und r c ntrol A typical xample of thi approach i th tudy of smoking di ordant twins with re pe t t IHD and lung fun tion (Lundman 1966)

I haemic heart disease in twins

Sev ral case rep rt hav been published con rning on ordance in MZ twin pair (Parade & Lehmann 1938; Froment t al 1945; B rna oni et al 1957; Benedi t 1958; Gikni et al 1965; Dougla 1966; Sidd et al 1966) but only a f w concerning di ordant pair (Sul r & Koller 1961; Le et al 1963) Although individual case reports on twin c n rdan for IHD obviou ly cannot prove th pre nce of h redity in IHD th y are sufficí ntly sugge tiv to rai a su pi ion

of its presence Kahler & Weber (1940) studied 17 twin pairs in which at lea t one of the twins suffered from IHD Three of the four MZ pairs were concordant with respect to IHD but only two of the DZ pairs Reviewing several small twin materials von Verschuer (1958) reported a combined IHD concordan e of 19 % in the MZ pairs as compared to 8 5 % in the DZ pairs

Only two representative uns lected twin sample the Danish and the Swedish Twin Regi tries are suitable for epidemiological studies

The Danish Twin Registry was founded in 1954 and ha been developed ever since (Harvald & Hauge 1968) It contains information on m dical and ocial conditions on about 8 000 unselected pairs of twins born in the p riod 1870 to 1910 where both partners had urvived the age of five By January 1 1968 coronary occlusion had occurred in 352 twins Th rate of concordance has been calculated by the twin proband method (Allen et al 1967) i e concordant pairs are counted twice as both partners are considered probands The concordance rate in the male MZ pair 39 % differed significantly from that of 26 % in the male DZ pair In the female pairs the difference was much great r 44 and 14 % re pe tively giving signi fi ance at a higher level The ri k of a co twin dying from coronary occlusion in th first 10 years after the proband s death was also calculat d For co-twin in MZ and DZ mal pair the risk curve w re very similar but for the female pairs they deviated considerably right from the start The risk of a female MZ co twin dying from oronary occlu ion was over 40 % in the first 10 year after th proband death as compared to about 4 % for a female DZ twin The corresponding risks for th male MZ and DZ pairs were about 15 % It wa conclud d that the occurr nce of fatal coronary occlu ion seemed t be genetically det rmined to a v ry limited extent in males and to a much larg r xtent in f males

In a qu tionnair tudy on the Swedish Twin Registry Cederlöf et al (1967) evaluated th prevalence of angina pectoris using th que tions work d out by Rose (1962) The inv stigation compri ed 2 255 MZ and 3 6?2 DZ twin pairs 40 to 80 years of age with concordant smoking habit The r ults show d a significantly higher degre of oinciden of angina pe tori in MZ compared to DZ pair for male a well a females in the ag group 60 80 year but for the younger age group 40 60 year thi wa tru only for the females Validati n of the mailed qu tionnaire con erning angina p toris (Lundman et al 1971) indicat that it i very us ful f r reening ca with IHD but on ha to be aware of a t ndency to giv false po itives

In a study of 196 smoking discordant male and female twins from the Swedish Twin R gi try Lundman (1966) evaluat d the occurrence of segmental ST depre sion of at lea t 0 5 mm at post-ex rcise el otrocardiography and found that the ob rved c incidenc rat wa ignificantly higher than the xpected rat in both MZ and DZ pairs It was suggest d that thes egmental change could be due to multiple dominant gen When the clinical pi ture of IHD wa in cluded in the judgement of IHD the difference within th MZ pair was more pron un ed but not within the DZ pairs which wa considered to indicat a sub tantial gen tic c mp n nt in IHD How v r female and male twin pairs were pooled in th e analy e ob tru ting furth r con lusion The occurrenc of IHD did not differ between moking and n n smoking twin pair

Liljefors (1970) studied 91 mal twin pair al o from the Swedi h Twin R gi try aged 42-67 year wh were o n ordant or dis- rdant with respect t IHD The con ordan e rate was calculated for diff rent manife tations of IHD F r myocardial infarction con cordance wa n in one MZ pair and on DZ pair Wh n the con pt of IHD was extended to includ angina pectori and/or pathological ele trocardiographi finding (Q_2 at re t ac ording to the Minne ota od and/or ST s gmental depre ions of at least 0 5 mm during xer ise) to indicat th probabl pr nce of IHD 48 5 % of the MZ pair were on ordant as compared to 28 5 % of the DZ pairs The diff ren e wa however not tatistically ignificant

Although diff rent manife tations of IHD hav b n mea ured the r ults of th e few twin studie do sugge t some general comment Th diff ren found in concordan rate b twe n MZ and DZ pair w re small r in mal s than female (f Harvald & Hauge and Cederlöf et al) Th e findings ould indi at that nvironmental factor are strong enough to outw igh or mask th g n tic influ nce in m n but not to the ame ext nt in women

Ba kground and obj tiv f the pre ent tudy

Th e tablishment of th Sw di h Twin R gi try provided a useful tool for pidemiologi al studie It wa t up primarily to study factors f tiologic importance f r di ea of th r pirat ry and ardio- va ular sy t m Th R gi try i admini tered by the Department of Environmental Hygi ne Karolinska Institute and a re earch program i carried out in ollaboration with th Department of Medicine at the Serafimer Hospital One lin of thi program is a c ntinuous m rtality foll w up an th r ompri clinical tudi s on sub ample

of the Twin Registry At the international twin symp ium in Puerto Rico in 1969 it was pointed out that no generally accepted methodology in the design of twin studies exi ts and there are many complex po sibilitie Studies of mortality wer considered of great imp rt ance and as valuable ext nsion clinical examinations of the partners of deceased twins wer suggested with a view to detecting any differ enc s in disease prevalence betwe n surviving MZ and DZ co twins No such studie have yet been carried out

From January 1971 information on deceas d twins has been avail able every month th reby permitting clinical xamination of the surviving co-twin reasonably oon after the death of the partner Thi provided one of the pre requi it for the present tudy By using a sample of unselected death di cordant twin th surviving co-twins could be examined in an unbia ed way with respect to variou manifesta tion of IHD and risk factor If an important genetic influen e on IHD exists it ems plausible that IHD ilent or manifest will appear significantly more often in the most gen tically predisposed twins i e MZ co-twin whose partner have died from IHD as ompared to DZ co twins whose partner have di d from IHD The comparison should also be extend d to the co-twins who e partner hav died from other cau e than IHD thus permitting a compari on b tween differ nt genetically predisposed surviving o twins The obje tive of the study can be ummarized a follows:

(1) Evaluate the genetic influence in IHD is ther an as ociation between the occurrence of IHD in the urviving co twin and the cause of death of th partn r (IHD or not IHD)?
(2) Is th re an a sociation b tw en the risk factor profile in the urviving co twin and the caus of death of the partner (IHD or not IHD)?
(3) Are there environmental diff rences in the death discordant pairs as elucidated from arlier questionnair s?

Furth rmore this tudy pr vid a basi for a continuous follow up of m rtality of the co twins examin d and thus makes it pos ible to a the pr dictive value of the mea ured ri k factors as well a th h r ditary influen e

MATERIAL

The mat rial of d ath di ordant twins for the pres nt inv tigation derive from the Swedi h Twin R gi try and the tudy onstitut part of the re ar h program for th R gistry (Fig 1)

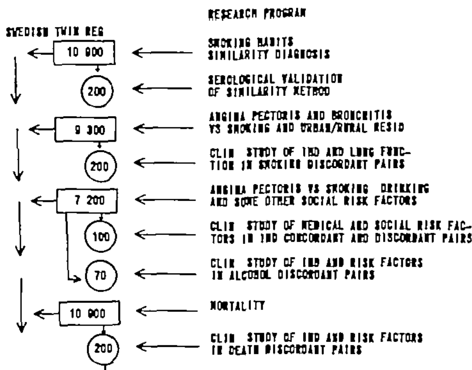


Fig 1 R arch program for the Swedi h Twin Regi try

Th Swedish Twin R gi try

Th Swedi h Twin R gi try wa et up in the y ars 1959 to 1961 at th d partment f Environmental Hygi n Karolinska In titut and th f rmer National In titut of Public H alth It ntains about 10 000 twin pairs and cov r about 95 % f all Swedi h same x twins who w re born in th country b twe n 1886 and 19 5 and with both member living wh n th R gi try was formed Th compilation procedure and th demographic tru ture of the twin eri have b n d scribed in detail by Ced rlöf (1966 a) Th Regi try has be n u ed for veral qu tionnaire tudi mainly d aling with r piratory symptoms and angina pe tori with spe ial ref renc to smoking (Cederlöf et al

1966 b; Cederlöf et al 1966 c; Cederlöf 1966 d; Cederlöf et al 1967 a; C derlöf et al 1967 b) and two clinical studies (Lundman 1966; Liljefors 1970)

Zygo ity diagnosis

Th zygosity of the twins in the Regi try has been determined with the aid of imilarity qu tions contain d in a questionnaire mailed to each one of them (Cederlöf 1966 a) One of th se items proved t be of high reliability namely th very simple qu stion whether the twins as children were as alike as two p a in a pod or of family likene only The qu stions were validat d by C derlöf et al (1961) on 200 randomly selected pairs by comparing th que tionnaire diagno is with blood-group rology in respect of five independent systems namely A_1A_2BO MN Rh Hp and Gm The MZ and the DZ diagnosis arrived at from the que tionnaire agr ed with th serologi diagn is in 99 % and 91 % respectively About 4 % of the twins gave confli ting answ rs con erning similarity and have therefore been classifi d as of unknown zygosity (XZ) Excluding the XZ group the MZ twins mak up 35 6 % of the men and 34 1 % of the women in the Registry The zygosity diagno in th pr s nt tudy are xclusively based on the diagno es earlier made in the Twin Regi try

Mortality evaluation

Th m rtality among the twin i e tabli hed as follows The total twin regi try i match d regularly against a total d ath regi try for Sw den at the Central Bureau of Stati tic Sinc 1971 thi ha b en done once a month (Boland r 1973) The procedure provides acces to the d ath certificate and the nam of th physician who signed it Th certificate in turn indicates wheth r or not the deceased twin had been treated in a h pital H pital record autop y re ords information from gen ral practition r and other p rtinent inf rma tion are colle t d The ause of death i then established from all these r cord tc The final evaluation ha been made together with two oth r do tor (Dr L Friberg and T Lundman) and the caus of d ath ha b en la ifi d ac ording to th 1965 revi ion of Inter national Stati tical Classification (ISC 1969) of Di a Injurie and Cause of Death whi h ha been us d ince 1969 in this country Furth r detail of th m rtality evaluation will be found in two report on the mortality follow up in moking di cordant twins (Friberg et al 1970 and 1973)

Criteria for electi n of the pre nt mat rial

During th peri d January 1st 1971 to March 15th 1973 262 male and female twin pairs b low the ag of 70 b ame d e a t h d i - o o r d a n t i e n of th member in an unbroken pair died during the p riod mentioned above About two or three months after death di c rdance had o curred the surviving co-twins were invited to a thorough health heck up at S rafimer Ho pital Stockholm Th el ction procedure i visualiz d in Fig 2

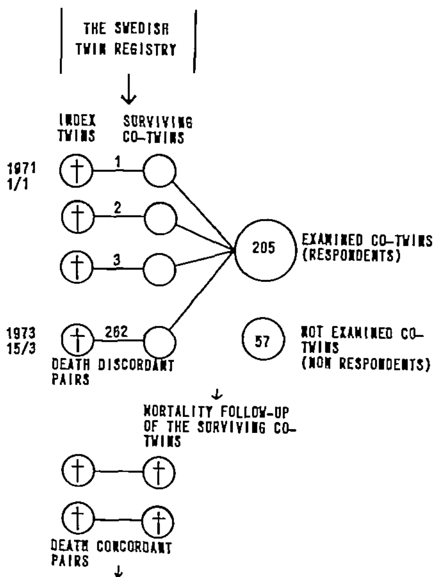


Fig 2 S l tion of th pr ent material

Table 1 Causes of death of th index-twins with a breakdown by sex

	Males		Females		B th sexes	
	No	%	No	%	No	%
All causes	141	100 0	121	100 0	262	100 0
IHD total	48	34 0	21	17 4	69	26 3
Myocardial infarction	34	24 1	10	8 3	44	16 8
Oth r IHD			2	1 7	2	0 8
Sudden death	14	9 9	9	7 4	23	8 8
Not IHD total	93	66 0	100	82 6	193	73 7
CVD	10	7 1	10	8 3	20	7 6
Malignant tumor	34	24 1	53	43 8	87	33 2
Uraemia	1	0 7	2	1 7	3	1 1
Diabetes mellitu	2	1 4	2	1 7	4	1 5
Suicides	8	5 7	4	3 3	12	4 6
Accident	8	5 7	8	6 6	16	6 1
Other aus	30	21 3	.1	17 4	51	19 5

The deceased twins

The de a d twins constitute the index twins in the death di cordant pairs A breakdown by cau e of death d termined a earlier described is given in Table 1 using the following group (ISC numb rs in parenth sis): IHD (410 00 414 99 795 99) cerebrovascular disease (CVD) (430 00 438 99) malignant tumor (140 01-209 99) uraemia (792 99) diab tes mellitus (250 00 250 09) suicid s (E 950 9 E 959 9) accident (800 00-999 99 E 807 0 E949 9 E 960 9 E 999 9) and oth r causes (conditions not specifi d here but corre ponding to th remaining ISC numbers) IHD here includes myocardial infarc tion (410 00-410 99) sudden death (795 99) and other IHD The la t heading contain only two f mal cases both classified as 412 09 (arterioscl ro is cordis NUD um hypertonia) Those death di cordant pair who e ind x twins hav died from IHD are in the following referr d to as I H D d e a t h d i s c o r d a n t Correspondingly tho e pairs wh se index-twins died from oth r caus than IHD are r f rr d to a n o t I H D d e a t h d i s c o r d a n t

Ho pital re rd or similar ources of information wer available in 224 out of th 262 deaths (85 5 %) For the remaining 38 deaths (14 5 %) only d ath certifi ate w re available but additional information wa ought in the ca e fr m relatives and/or th surviving co twin An autop y had b n p rformed in 149 of the de eas d twins or 56 9 % Th ca referred to autopsy includ d 35 forensic post mortems (13 4 %)

The IHD cases included 23 cases of sudden death of which 16 (69.6 %) occurred within two hours after the onset of acute symptoms. No exact information was available about this interval in the other 7 cases.

From the collected hospital records, autopsy protocols, etc., an assessment was also made of whether the deceased twin had a pre-existing history of IHD and/or autopsy findings indicating IHD. A pre-existing history was considered most probable if one of the following two diagnoses had been present:

(1) Myocardial infarction confirmed at hospital or evidence of old infarction in the resting ECG.
(2) Angina pectoris on effort.

As autopsy findings indicating IHD were regarded the existence of fresh or old infarcts of the myocardium and/or marked arteriosclerotic changes (uncuttable arteries and/or occlusions) of the coronary arteries. Thus, of the 23 cases of sudden death, 16 (69.6 %) had either a pre-existing history of IHD or a positive autopsy. The corresponding figures for the 193 cases not assigned to the IHD group were 7 (3.6 %).

The surviving co-twin

The first twins were examined in June 1971 and the last in the beginning of August 1973. At the time of the check-up the investigator (the author in all cases) was aware neither of the index-twin's cause of death nor of the zygosity.

Respondent co-twins

The response rate and causes of non-response are given in Table 2. Of 262 twins invited 205 were examined, which gives a response rate of 78.2 %. Among those examined, 14 were unable to come to Stockholm but were willing to attend a hospital nearer their home. Of these 3 men and 3 women were examined at Sahlgrenska Hospital in Gothenburg; two of them came from places in the County of Bohuslän north of Gothenburg, the rest lived in Gothenburg. Another two men were examined at the Central County Hospital of Uddevalla and two women at the Central County Hospital of Lidköping. One woman and one man were examined at the County Hospital of Bollnäs, one man at the Central County Hospital of Karlstad and another man at the County Hospital of Finspång.

Tabl 2 Re ponse rat e and auses of non-resp n by ex and zygosity

	MZ		DZ		XZ		Tot	
	No	%	No	%	No	%	No	%
Males:								
Total in sample	41	100 0	94	100 0	6	100 0	141	100 0
Total xamined	35	85 4	67	71 3	6	100 0	108	76 6
Examined in Stockholm	34	82 9	61	64 9	5	83 3	100	70 9
Examined in the provino	1	2 4	6	6 4	1	16 7	8	5 7
Not examin d: total	6	14 6	27	28 7	0	0 0	33	23 4
No conta t	1	2 9	2	2 1	0	0 0	3	2 1
Illne	2	4 9	7	7 4	0	0 0	9	6 4
R fu al owing to work	1	2 4	7	7 4	0	0 0	8	5 7
R fusal f r other rea ons	2	4 9	8	8 5	0	0 0	10	7 1
Data losses	0	0 0	3	3 2	0	0 0	3	2 1
Females:								
Total in ample	47	100 0	72	100 0	2	100 0	121	100 0
Total xamined	38	80 9	57	79 2	2	100 0	97	80 2
Examin d in Stockholm	35	74 5	54	75 0	2	100 0	91	75 2
Examined in the provinces	3	6 4	3	4 2	0	0 0	6	5 0
Not examined: total	9	19 1	15	20 8	0	0 0	24	19 8
No ontact	2	4 3	0	0 0	0	0 0	2	1 7
Illnes	3	6 4	2	2 8	0	0 0	5	4 1
Refu al owing to work	1	2 1	2	2 8	0	0 0	3	. 5
R fu al for other reasons	2	4 3	10	13 9	0	0 0	12	9 9
Data losses	1	2 1	1	1 4	0	0 0	2	1 7
Both sexes:								
Total in sample	88	100 0	166	100 0	8	100 0	26.	100 0
Total examined	73	83 0	124	74 7	8	100 0	.05	78 2
Examined in Stockholm	69	78 4	115	69 3	7	87 5	191	72 9
Examined in th provinces	4	4 5	9	5 4	1	12 5	14	5 3
Not examined: total	15	17 0	42	25 3	0	0 0	57	21 8
No contact	3	3 4	2	1 2	0	0 0	5	1 9
Illness	5	5 7	9	5 4	0	0 0	14	5 3
Refusals owing to work	2	2 3	9	5 4	0	0 0	11	4 2
Refu al for oth r reason	4	4 5	18	10 8	0	0 0	22	8 4
Data los e	1	1 1	4	2 4	0	0 0	5	1 9

Tabl 3 Di tributi n f re pond nt co twin (by x and ygosity) according to au of d ath f ind x twin

	NUMBER OF RESPONDENT CO-TWINS											
	Mal				F mal s				Both x			
	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	T t
All c u	35	67	6	108	38	57	2	97	73	1–4	8	05
IHD t tal	10	5	5	40	8	9	0	17	18	34	5	57
My ardial infarcti n	8	18	4	30	3	5	0	8	11	3	4	38
Oth r IHD	0	0	0	0	1	1	0	2	1	1	0	–
Sudd n d ath		7	1	10	4	3	0	7	6	10	1	17
Y t IHD total	5	4	1	68	30	48		80	55	90	3	148
CVD		4	0	6	–	5	0	7	4	9	0	13
Malignant tumor	9	18	0	27	19	1	–	4	8	39	–	69
Ura mia	1	0	0	1	0		0	–	1		0	3
Diab te m llitus	0	1	0	1	0	1	0	1	0	–	0	
Sui id s		3	0		1		0	3	3	5	0	8
A cid nt	1	6	0	7	1	5	0	6		11	0	13
Oth r cau	10	10	1	21	7	1–	0	19	17		1	40

Table 4 Distribution of mean age (y ars) of re pondent co twins (by sex and zygosity) according to cause of death of index twin

	Mal				F males				Both exes			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
All au	61 1 (1 1)	60 4 (0 8)	58 0 (3 2)	60 5 (0 6)	59 7 (1 1)	61 5 (0 8)	60 5 (1 5)	60 8 (0 6)	60 4 (0 8)	60 9 (0 5)	58 6 (2 4)	60 7 (0 4)
IHD t tal	57 7 (1 8)	63 0 (0 8)	57 2 (3 8)	60 9 (0 9)	63 3 (2 2)	64 9 (1 8)	0	64 1 (1 4)	60 2 (1 5)	63 5 (0 8)	57 2 (3 8)	61 9 (0 8)
Myo ardial infarction	59 0 (1 9)	63 7 (1 0)	59 5 (4 0)	61 9 (1 0)	57 7 (4 2)	62 6 (2 9)	0	60 8 (2 4)	58 6 (1 7)	63 5 (0 9)	59 5 (4 0)	61 7 (0 9)
Other IHD	0	0	0	0	65 0 (0 0)	69 0 (0 0)	0	67 0 (2 0)	65 0 (0 0)	69 0 (0 0)	0	67 0 (2 0)
Sudd n death	52 5 (3 5)	61 0 (1 3)	48 0 (0 0)	58 0 (1 9)	67 0 (1 2)	67 3 (0 9)	0	67 1 (0 7)	62 2 (3 3)	62 9 (1 3)	48 0 (0 0)	61 8 (1 6)
Not IHD total	6 5 (1 3)	59 0 (1 1)	62 0 (0 0)	60 3 (0 8)	58 8 (1 2)	60 9 (0 8)	60 5 (1 5)	60 1 (0 7)	60 5 (0 9)	60 0 (0 7)	61 0 (1 0)	60 2 (0 5)

The distribution of the places of re idence of the respondent co twins is shown in Fig 3 The places of resid nce are scattered over mo t of Sweden and em to correspond fairly well with the population d nsity

In Table 3 the examined co-twins are distributed ac ording to the cause of death of th index-twin There were 57 (27 8 %) who e partners had died from IHD i e 40 of th 108 male pairs (37 0 %) and 17 of the 97 female pair (17 5 %) Sudd n death caused 25 % of the IHD deaths among the male and 41 2 % among the female It is al o worth noting that of th 8 pairs of unknown zygo ity (XZ) as many a 5 were male IHD death discordant

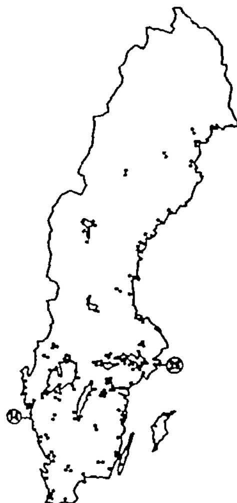


Fig 3 Di tribution of th pla e of residence of the re pondent twins Th figure in th two circles indicate th number of co-t living in Stockh lm and Gothenburg

The distribution of th respondent o twins by 5-year age groups is shown in Fig 4 and Table 4 gives the means for age by the cause of death of the index-twin

The mean age at th time of d ath discordance for all the respondent co twins was 60 7 years Among the IHD death dis ordant pair the mean age of the male MZ pair 57 7 years wa on average 5 3 years lower than that of the corresponding DZ pair The male MZ twins whos index twins had died from causes other than IHD had a mean age of 62 5 years compar d with 59 0 years for the corresponding DZ pairs The mean ages of the male XZ pairs in the different death discordant group are about the ame as for th male MZ pairs Among the female IHD death discordant pairs the mean ages for both th MZ pairs and the DZ pair 63 3 years and 64 9 years respectively are 4 5 and 4 0 years higher than for the MZ and DZ pairs whos ind x twins died from oth r cau es than IHD

The surviving co twins were examined on average about 5 months after the death of th index twin (Table 5) th interval ranging b tween two and 12 months

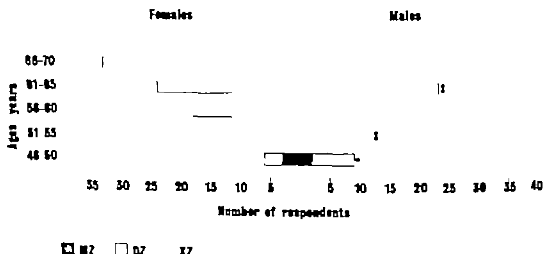


Fig 4 Distribution of re pondent o twin by 5 year age group

Table 5 Distribution of int rval (m an and rang in months) between death of index-twin and examination f co-twin a cording to caus of d ath of index twin

Cau e of death of the ind x twin		MZ	M Range	DZ	M Range	XX	M Range	Tot	M Rang
Mal	IHD	n=10	4 9 4 0 6 0	n=25	5 5 2 5-12 0	n=5	4 0 3 0-6 0	n=40	5 2 2 5 12 0
	not IHD	n=25	5 6 3 0-12 0	n=42	5 3 2 0 11 0	n=1	9 0	n=68	5 5 2 0-12 0
Female	IHD	n= 8	5 3 3 0-9 0	n= 9	5 6 3 0 10 0	n=0		n=17	5 4 3 0-10 0
	n t IHD	n=30	4 8 2 0 8 0	n=48	5 0 2 0 11 0	n=2	5 3 5 0 5 5	n=80	5 0 2 0-11 0
Both x s	IHD	n=18	5 1 3 0-9 0	n=34	5 5 2 5-12 0	n=5	4 0 3 0 6 0	n=57	5 2 2 5-12 0
	not IHD	n=55	5 2 2 0 12 0	n=90	5 2 2 0 11 0	n=3	6 5 5 0-9 0	n=148	5 2 2 0-12 0

Non re pondent co twins

Th re wer 57 twins who did not participat in th tudy (Table 2) Of th e 5 ould not be traced in spit of attempt by letter and t le phon Another 14 were unabl t parti ipate owing to illne s Two of th m b th men wer pati nts at m ntal h spital while two other men w r patient at long-t rm clini On of the latter (MZ) di d howev r in M rbus Altzheimer only 7 months after th ind x-twin The cau of death attributed to the ind x twin was r bro va cular di as On female and one male twin w r unable to participate b aus of invalidity aft r cer bral haemorrhage; th mal twin (DZ) di d from a myo ardial infarction about 8 month aft r the death of th index twin which had b n caused by anc r of th stoma h Anoth r male twin (DZ) could not att nd th examination becaus of g n ral fatigu and weakn He died later from a bron hopn umonia about 16 months aft r the ind x twin for whom the cau was cancer of the pro tat gland An un pe ified neurological di order mad parti ipation impo ible for anoth r male twin Two twins w re tied to wh l chair on woman b caus of invalidity after poliomyelitis and n man becau of a marked g n ral rh umatoid arthriti One

female and one male twin had recently been operated for gallbladder disease and cerebral malignant tumor re pectively Finally on woman was convalescent after a fracture of the femur and another was unable to participate becau e of general fatigue; she also suffered from hyperten ion

Lack of time on account of work was the rea on given by 11 twins 8 male and 3 female for refusing to participat They were all offered an examination at their home ho pital Among th other refusal were 6 twins who declar d a negative attitude towards health che k ups in general The remaining 16 twins in this group supplied no definite reason for not participating

As already mention d the total Twin Registry is matched regularly with a total death registry for Sweden; since 1971 this i done every month The procedure involves a slight risk of incorr t matching Because of this the Twin Regi try has b n match d twice so far with the total registry of living people in the country The fir t time was in January 1971 and the se ond in September 1973 At the last ch ck which covered the time of th present inve tigation it was found that 5 of the twins had been incorrectly matched They are li ted in the table as data lo ses

A breakdown of the non respondent co twins by the cause of death of the index twins i giv n in Table 6 There w re 12 non responder (21 1 %) whose partners had died from IHD which i a somewhat low r proportion compar d to the respondent group; this is especially true for the mal It will also be seen that ther are no XZ pairs among th non respond nts Furth rmore the percentage of MZ (the XZ omitted) among the males i le than for the respondents 18 2 and 34 3 re pectively Among the females th re are no sub tantial differences of thi kind

Figure 5 show the distribution of the n n-r ponders by 5-year age gr up and Table 7 the m ans for age by the cau e of d ath of the index-twin

Th mean ag of the male IHD death discordant pair 61 3 years is only 0 4 y ars high r than the m an for the corresp nding group of respondent males Howev r th non responding male co twins (only DZ pair) who e index twin died from myocardial infarction are on average 7 4 years younger than th corre ponding male DZ twins among the responders 56 3 year and 63 7 year re pectively The oppo ite age trend i seen in the non re ponding male co twin who e index twin died from sudden death (MZ + DZ pair) the mean age 66 3 y ars being on average 8 3 y ars high r than that of the corre ponding respondents

Tabl 6 Di tribution of non r p ndent co-twin (by x and zygosity) according to cau e of d ath of index twin

	NUMBER OF NON RESPONDENT CO-TWINS											
	Male				Females				Both sexes			
	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot
All au	6	27	0	33	9	15	0	24	15	42	0	57
IHD total	1	7	0	8	2	2	0	4	3	9	0	12
Myocardial infarction	0	4	0	4	1	1	0	2	1	5	0	6
Oth r IHD	0	0	0	0	0	0	0	0	0	0	0	0
Sudd n death	1	3	0	4	1	1	0	2	[illegible]	4	0	6
Not IHD total	5	20	0	25	7	13	0	20	12	33	0	45
CVD	2	2	0	4	1	2	0	3	3	4	0	7
Malignant tumors	2	5	0	7	3	8	0	11	5	13	0	18
Ura mia	0	0	0	0	0	0	0	0	0	0	0	0
Diabet s mellitus	0	1	0	1	0	1	0	1	0	2	0	2
Suicid	0	3	0	3	1	0	0	1	1	3	0	4
Accid nt	0	1	0	1	1	1	0	2	1	[illegible]	0	3
Oth r caus	1	8	0	9	1	1	0	2	[illegible]	9	0	11

Table 7 Di tributi n of m an age (y ar) of n n r spondent co-twin (by sex and zygo ity) according to au e f d th of index twi

	Mal MZ M (SE)	Mal DZ M (SE)	Mal XZ M (SE)	Mal Tot M (SE)	Females MZ M (SE)	Females DZ M (SE)	Females XZ M (SE)	Females Tot M (SE)	Both sexes MZ M (SE)	Both sexes DZ M (SE)	Both sexes XZ M (SE)	Both sexes Tot M (SE)
All au	64 5 (1 [illegible])	60 8 (1 [illegible])	0	61 5 (1 1)	59 [illegible] ([illegible] 1)	61 6 (1 5)	0	60 8 (1 2)	61 4 (1 5)	61 1 (1 0)	0	61 [illegible] (0 8)
IHD total	6[illegible] 0 (0 0)	61 1 ([illegible])	0	61 3 ([illegible] 3)	6[illegible] 5 ([illegible] 5)	53 5 (4 5)	0	58 0 (3 7)	6[illegible] 3 ([illegible] 6)	59 4 ([illegible] 4)	0	60 2 (1 9)
My ardial infarction	0	56 3 ([illegible] 4)	0	56 3 ([illegible] 4)	58 0 (0 0)	49 0 (0 0)	0	53 5 (4 5)	58 0 (0 0)	54 8 ([illegible] [illegible])	0	55 3 ([illegible] 0)
Oth r IHD	0	0	0	0	0	0	0	0	0	0	0	0
Sudden d ath	6[illegible] 0 (0 0)	67 7 (0 9)	0	66 3 (1 5)	67 0 (0 0)	58 0 (0 0)	0	62 5 (4 5)	64 5 (2 5)	65 3 ([illegible] 5)	0	65 0 (1 7)
Not IHD total	65 0 (1 3)	60 6 (1 5)	0	61 5 (1 3)	58 4 ([illegible] 5)	6[illegible] 8 (1 3)	0	61 3 (1 3)	61 [illegible] (1 8)	61 5 (1 1)	0	61 4 (0 9)

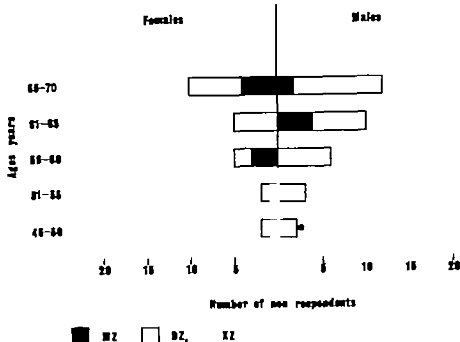


Fig 5 Di tribution of n n re pond nt by 5-y ar ag group

Comment

On of our b t measures of th reliability of mortality tati tic i pr bably a high autopsy rate (Britton 1974) In 1968 th autopsy rat in Sw d n was about 45 % of all d ath while for the age pan 1 64 y ar it amounted to about 50 % (Vedin t al 1971) which c rre pond fairly well with the figure of about 57 % in th present mat rial For a urat m rtality evaluati n however an autopsy rate of 57 % m rath r low Thi i unf rtunat ly a problem for all kind of mortality f llow up which include p pl living out id the univer ity iti in Swed n

Th d ath of th index twin ha been las ified in 23 ase as sudd n and un xpe ted and be n included in th IHD group The d finition of udd n d ath vari nsid rably An int rnati nal ommittee ha agr ed upon a d finition to fa ilitat int rnational comparisons udd n d ath b ing d fined a d ath o curring immediat ly or within an timated period of 24 hour aft r the ons t of acut obj ctive and subj tiv ymptoms (Paul & Schatz 1971) Oth r time criteria ar al o commonly u ed g one hour (Chiang et al 1969 and 1970; Gord n & Kann l 1971) r two hours (Ad l on L 1961; Kuller et al 1966) In a study of 967 medically unatt nded d aths from IHD o curring

in Stockholm during one year (Wikland 1971) information concerning the time relationship b tween the onset of ymptoms of the fatal attack and death was obtained in 62 % Of these 61 % of the male and 48 % of the female case died within 15 minute of onset of symptoms The definitions of sudden death have been carefully analysed in an editorial in Circulation (Biörck & Wikland 1972) It was pointed out that the a curacy of a statement of sudden d ath u ually depends on the observer and from that point of view it i important to note whether th preceding attack was witnessed or not (by any ob erver) It was furthermore conclud d that for unwitn ed deaths it is difficult to know how sudden death occurr d but a omparable propor tion of these ca es can usually be estimated to have o curred within the same tim intervals as the witne s d on

About 70 % of the sudden deaths in the pre nt investigation occurred within two hours and were witn ssed 30 % were unwitne sed and occurr d without exact information of time but probably within 24 hour Th per ons who died suddenly in hospital with signs of acute myocardial damage on the ECG or at autopsy have b en cla sified not as sudden death but a myocardial infarction Nor were thos per ons included among th sudden deaths who died suddenly but with strong indications of oth r probable explanations for th caus of d ath It is well known that especially with the 24 hour interval other cau es than IHD may have been r ponsible for death as for instanc major sudden cerebrova cular hemorrhage pulmonary embolism asthma bronchiale and rheumatic h art di ea es e pecially aortic st nosi (Kuller 1966; Mob rg 1969; Wikland 1971; Vedin et al 1973)

Of the 23 index twins cla ified as sudden deaths 16 (69 6 %) had eith r a pre xisting history of IHD or autop y finding indicating the pr nc of IHD Of th e 16 11 were among the 16 (68 7 %) who died within two hour and 5 w re among the 7 (71 4 %) who died within 24 h ur In the 7 of the 23 (30 4 %) without earlier evid n e of IHD it se med rea onable to suppose how ver that the sudden d ath wa m t probably the initial clini al manife tation of previously unknown IHD Thi is in accordanc with earlier report which point to incidences of about 20-25 % (Kuller et al 1966; Kuller 1966)

On of th problems in pidemiological studi i the lack of compl t parti ipation Thi difficulty i of ourse more pronounced if the populat on und r study i drawn from the whole of Swed n in tead of a more r trict d area The pre nt re ponse rate of 78 2 % can be c mpared with a rate of 79 4 % in a co-twin control study of 196 smoking di cordant twins by Lundman (1966) and 87 5 % respons in

a study of IHD in 91 twin pair by Lilj fors (1970) In the two latter tudi ther were some geographical re trictions with respect to th residence of th twin pair Lundman took hi twin ampl from everal towns in the central part of Sw d n and Liljefors obtained hi DZ pairs from 10 countie in central Swed n There w re no such r trictions in the sel ction of twin pair in th pr nt study Furth rmore the mean age of th present twins was 60 7 years which i about 3 year higher than in Liljefors tudy and about 10 years higher than in Lundman a circumstance which might al o tend to low r th re ponse rat

In population studi concerned with a particular di ase it has been r cognized that per ons with the disease in qu stion are more likely to participate than oth rs (Cobb et al 1957) Th twin pair in th pre ent inv tigati n w r a cordingly not informed that the primary intention wa to study IHD They w re told that thi was a gen ral h alth heck up c nstituting part of th re arch program on th Swedish Twin R gi try and furthermore that only th surviving o twins in death di ordant pair w re included

A non-participation group i often thought to differ in various way from th group xamined In a population tudy of 973 50-year-old men (Tibblin 1967) an analy i of the n n participant (Tibblin 1965) failed to show that th y wer mor sick than tho e examined It was al o f und that th chief r a on for non parti ipation (38 %) was a negative attitud to medical care in gen ral In the pre ent inve tigation the larg st group f non re ponse (22 out of 57) ha been labelled r fusal for oth r rea ons This in lude 6 per on who frankly d lar d a n gativ attitude to d ctor hospital and h alth ch ck up Th r t of thi group (16 p rsons) gave no r ason for their refusal

As regard ag ex and zygo ity th r ar ome differen b twe n th re pondent and non respond nts Th mal MZ twins cl arly show a greater willingn to parti ipate than the DZ male and thi readin ems t be ind p nd nt of th partner ause of d ath Ther is no su h diff r n among the female twins The male twins who did n t parti ipat were al o on av rage one year older than th r pond nt It i of cour e difficult not to ay impo sibl to t ll wh th r th ob rved differ nce an influ nce a valid ompari n betwe n th zygo ity groups Howev r th preval nc of IHD a e timat d from an arlier qu stionnaire did not rev al any ub tantial diff r nc betwe n the respond nt and n n re pondent

METHODS

Time schedule for th examinations

The first oo twins to be examined were seen in June 1971 and the last 26 months later in Augu t 1973

The twin arriv d at about 8 00 a m for the examination which was completed by 4 00 p m on the ame day One or two twins a day were xamin d The procedures were undertaken ac ording to a fixed time schedule

Questionnaire and interview

The twins were all intervi wed by the author in accordance with special questionnaires including question on earlier diseases hospital treatment and medication as well a questions on sociologic data smoking habits use of alcoholic beverag s The questions on cardio-va cular symptoms followed a questionnaire d igned at the London School of Hygien and Tropical Medicine (Rose 1962) The questionnaire on respiratory symptoms was ba ed on that worked out by the British M dical Research Council (1960)

Physical examination

A routin clinical bedside examination was p rformed on every twin supplem nted with the following examinations

Blood pres ure measur m nt

The blood pre ur wa measured in supine po ition by the author onc at the beginning of the xamination (ca ual blood pressure) and th n aft r at lea t 15 minutes r t (ba al blood pressure) A mercury manomet r was u ed with a sleeve mea uring 13 x 40 cm and th standard procedure re ommended by WHO (1968) was followed Th blood pres ur wa read to th nearest 5 mm Hg Th ystolic pres ure level wa det rmined by the first per eption of ound The dia tolic fourth pha e l vel wa r corded when th ounds w re fully muffled and th diastoli fifth phase wh n th sound disappeared

Anthropometri m a urem nt

W e i g h t wa mea ur d to the nearest kilogramme with the subj ct

dressed in undershorts

H i g h t was measur d to th nearest entimetr with the subjoot barefo ted and his back again t the wall

S k i n f o l d t h i o k n e s wa measured with a Harpend n caliper (Edward et al 1955) giving a pre sure of 10 g/mm^2 in th subscapular and triceps ar a on th right side of th body Two m a urements were p rformed at each site and th mean was recorded to the nearest 0 2 mm

R e l a t i v e w e i g h t wa determined ac ording to a weight/ height index $\frac{\text{weight (kg)}}{\text{height (cm)} - 100} \times 100$

A r m o i r o u m f e r e n was measured to the n arest 0 5 centimetre at the mid of the p ndant un loth d right upper arm

El trocardiographi xamination

ECG were recorded at re t and for n arly all of the ubj cts also during and aft r an ex roi te t on a bi yole rgomet r The record ing w re made after about 10 minut s r t in upin position b fore exeroi e and with the following leads: I II III aVR aVL aVF CR_1 CR_2 CR_4 CR_5 and CR_7 During x roise with the ubje t itting on a bicycle registrations wer p rf rmed aft r 2 4 5 and 6 minutes at each load with the leads CH_2 CH_4 CH_5 and CH_7 (Holmgr n & Strandell 1961) Th exeroi t t start d gen rally at a load of 300 kilopond-meter/min (kpm/min) f r both men and women The l ad was incr as d at pwi e after th completion f ach 6 min peri d for men by 300 kpm/min and for women by 150 kpm/min 1 3 and 10 minute after xeroi e ECG wer again r o rded in supin po ition with th foll w-ing leads: I II III CR_1 CR_2 CR_4 CR_5 CR_7

A dir t writing 4 hann l l otrocardiograph was us d on th ubje t xamined in Stockholm (Mingograph 42 Elema-Schönand r AB Solna Sweden); tho in the provinc w r m stly xamined with a dir t writing 6 channel l trocardiograph (Mingograph 61 Elema S hönander AB S lna Swed n) A mechanically braked bicycl ergom ter (Monark Cr entbolagen AB St ckholm Swed n) was u ed the same for all th ubj t but n examined in Karl tad where an electro dynamically brak d bicy le rgomet r was u ed (Elema Schönand r Solna Sweden) Th paper sp ed for th regi trations wa 50 mm/s o before and after th t st and al o during th 5 min registrations at each ex roi load The other re ording during ex rci e were performed with a pap r speed f 25 mm/ c Th heart rate wa calculated from th ECG

The test was stopped when the patient was unable to continue due to fatigue dyspnoea angina pectoris or marked ST segment d pression or serious arrhythmia In some case the test was discontinued earlier for orthopaedic rea ons

W o r k i n g c a p a c i t y The maximum working capacity (W_{max}) was calculated according to a formula of Strandell (1964) To the greatest load the subject wa able to perform for 6 minutes (W_6) was added n x W_d/6 kpm/min where n i the number of minutes at th final load and W_d the difference between the two highest loads

C o d i n g The ECGs were all coded by the author according to a modified system (Åstrand et al 1967) of the original Minnesota Code (Blackburn et al 1960)

The codings w re made by th author over a period of about one month after completion of the study All the ECGs were checked twice and some were also interpreted by an independent observer (Dr T Lundman)

Radiologic examination

Radiologic examination of heart and lungs was performed on every ubj ct The exposures w re tak n with the subject er ct and in two plan (frontal and lateral) The tube-film di tance for both posi tions was 1 5 m at all examinations An experienced radiologist (Dr G Skogsberg) xamined all the films and determined the total and relativ h art volume (Jonsell 1939)

Blood investigations

At about 8 00 a m venous blood samples were drawn after about 12 hour fast and thir t All the analyses on blood from the subje ts examined in Stockholm were made at the Departm nt of Clinical Ch mi try S rafimer Hospital In the case of subjects examined out sid Stockholm erum was obtained and transported in thermoses with carb nic acid snow for later analy is of cholesterol triglyc rid s and uric acid at the Department of Clinical Chemi try Serafimer Ho pital

C h o l e s t e r o l wa d termined in an autoanalyzer according to the modifi d meth d of Levine & Zak (1964)

T r i g l y e r i d e w re determined in an autoanalyzer by the method of Kobl & Campbell (1970)

U r i c a c i d wa determined in an autoanalyzer by the method of Hawk et al (1954)

The methods were checked c ntinuously throughout the study by d terminations on standard erum

The other blood te t included fasting blood sugar hemoglobin venous hematocrit erythrocyte sedimentation rate (E S R) a well a qualitative tests for urin protein and urine glu ose The determinations were made at the hospital in qu tion in ac ordance with th routin methods us d there

Statistical method

Continuous and discrete variable among the surviving co-twins wer compared according to the heme in Fig 6

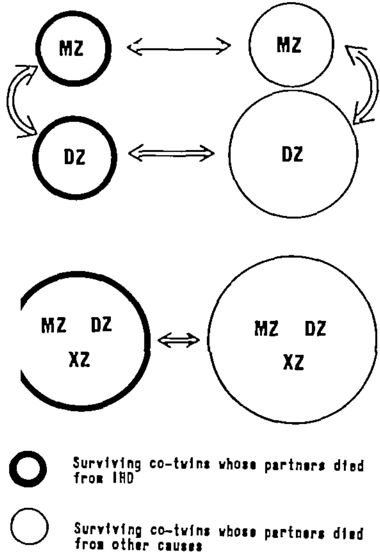


Fig 6 Stati tical compari ons between the various group of surviving co-twins

Conventional methods were used for th calculation of mean values (M) and standard error of the mean (SE) Significance of diff rences between mean values was tested by Student s t-test Differences between proportions were tested in fourfold tables by Fisher s exact probability test Differences between combinations of risk fa tors (Chapter III ction 4) were test d in two-by-k tables using the chi-square test

Intra pair difference (the death discordant pairs) of qualitativ variable such as moking and register d abu e of alcohol (Chapter III, section 3) wer examin d by the use of tabl s of binomial probability distribution (McNemar 1955) If the direction of the distribution could be expected the one tailed probability wa calculated Degrees of significanc were te ted at the levels of 5 % ($p<0$ 05*) 1 % ($p<0$ 01**) and 0 1 % ($p<0$ 001 ***)

III

RESULTS

1 ISCHAEMIC HEART DISEASE AMONG THE SURVIVING CO-TWINS

Thi oti n deals with th pr val n e of angina p otoris myocardial infarcti n and lectrocardiographi chang s sugg tive of IHD among th surviving co twins

Mat rial and Method ee Chapter I and II

Que tionnaire and ca hi tory finding

The distribution of angina pectori a record d by interview a c rding to th qu stionnair proposed by Ros (1962) and that of myo cardial infar tion verified at h pital are giv n in Table 8

Table 8 Di tribution of angina pectori and myocardial infarcti n (ab lute numb rs and preval nce rat) among r spondent c twins a ording to cau of d ath of index twin

		IHD								n t IHD							
		MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
		No	%	No	%	N	%	No	%	No	%	No	%	No	%	No	%
Male	Angina pect ri	1	10	2	8			3	7		-	1	2	-	-	1	1
	Myocardial infarction	3	30	3	12	-		6	15	1	4	1	2		-	2	3
	Total number	10		5		5		40		5		42		1		68	
Femal	Angina p toris	2	25	1	11	-	-	3	18	-		4	8	-	-	4	5
	Myo ardial infar ti n		-		-	-	-		-	-		1	2	-	-	1	1
	Total numb r	8		9		0		17		30		48		2		80	

The preval nce of angina p toris and myo ardial infarction wa higher among the male co twin who e partner had died from IHD (7 % and 15 % respectively) than among thos whose partn r had one other cause of death (1 % and 3 % re pectively); among the zygo ity groups the highe t prevalenc rat w re found for the MZ co twins whose partners had died from IHD (10 % and 30 % re pectively) Among th female ubjects th prevalence of angina pectori was likewise higher for the co twin wh partners had died from IHD (18 %) compared to thos wh se partn r had died from other cause (5 %) None of the differen e wa stati ti ally ignificant Only on (1 %) of the femal had had a myocardial infarction v rified at hospital

Comment

The diagn sis of angina pectoris e tabli hed by interview ac ording to th questionnaire by Ro (1962) ha been found to have a high specificity and al o a fairly good s nsitivity when compared to linical judgem nt (Ro e 1962; H yd n et al 1971) How ver some pati nts diagno ed as having angina pe tori how normal c ronary arteriograms (Likoff et al 1967; K mp et al 1967); thi ha b n found p ially am ng premenopausal wom n (Fowler 197.) The r ported pr val n e of angina p ctori i about the same in men and women in sev ral tudies (Kannel et al 1961; Kann l & Feinl ib 197 ; B ngt on 1973) but the progno i a to urvival and progr si n t more eriou c ronary manifestation i parti ularly discouraging in m n The Framingham tudy h wed that on in 4 men with angina pe toris an expe t a c ronary attack within 5 year mpared to half the ri k for women (Kann l & F inl ib 1972) With regard to my cardi l infar tion the prevalen i consid rably higher among men (Da ber et al 1957; Ep t in et al 1965; Hag rup & From Hansen 1968)

In th pr nt tudy the prevalen e of angina pectoris i high r among th women than th men whil th r vers i true of myo ardial infar tion Th surviving male co twin who e partn rs di d from IHD, di play the highe t prevalen e of symptoms ugg tive of manifest IHD and th rate i p ially high among the MZ co twin Thi tend n y i mo mark d fo myo ardial infar tion v rified at h pital The ame t nd ncy appli to th wom n a regard angina p ctoris Non of th e t nd n ie however gave tati ti ally ignifi ant differ nce Owing to th r latively low preval n e of manif st IHD and the mall numb r of MZ and DZ o twins who partn r died from this cause it i diffi ult t a th significan of h redity in

manife t IHD It has be n said (Ep tein 1964) that manif st IHD r pr nt only th top of the iceberg and that the additional cases hidden b n ath the urface account for the relativ insensitivity of diagn sti instrument H wever ECG during and after ex ercise is a relatively ensitive instrument for the detection f sil nt forms of IHD and i probably th b st meth d in thi r spe t for epid mio l gical tudies (Blomqvist 1971; Helfant et al 1973; Åstrand 1973)

ECG and X ray findings

Th di tributions of certain ECG findings at rest and during or aft r x r i e ar hown in Table 9 and 10 Th findings have b en tabulated ac ording to the cod numbers in th modified Minnesota ode (Åstrand t al 1967) ST d pre sion T wave negativity and e topic beats were not coded in the 15 ubjects who w re on digitali therapy Resting ECG was recorded in all the co twins but ex rci e te ts c uld not be p rformed in 11 for variou rea ns (Table 11) The means for maximum w rking capa ity (W_{max}) and final heart rat in th co twins not treated with digitalis ar giv n in Table 12 Both parameters are lower on av rage for the co twin wh partn rs had died from IHD compared to tho whos partn rs had di d from oth r causes Among th mal MZ o-twin however both W_{max} and final h art rate are high r for th s with partners who died from IHD while among the mal DZ c twins th y are high r f r tho wh partn r died from oth r au than IHD Th differenc wer n t tati ti ally signifi- ant Part f th intra ygo ity differ nce is probably due to th diff r n s in ag Tabl 13 h ws th mean valu f r relative heart volume among th urviving o-twin For t hnical rea ons and/or pleural change no data ar availabl f r 3 femal and 1 male DZ co twins who e partner died from oth r caus than IHD Th male MZ co twins wh partn r died from IHD have larger relative heart volume than th ir mal DZ ount rpart ($p < 0.05$) but th pooled male zygo ity groups (IHD vs not IHD) do not diff r On th oth r hand the p led f male zygosity group (IHD v not IHD) differ ignificantly ($p < 0.01$) Th femal MZ co twins with partn rs who died from IHD hav ignificantly ($p < 0.05$) larger relative h art volume than the MZ o-twins who e partn r died from other causes There i a similar tendency among th DZ f males but the differenc is not significant

Table 9 Distribution of electrocardiographic changes at rest among respondent co-twins according to cause of death of ind x twin

		Male								Females							
		IHD				not IHD				IHD				not IHD			
	ECG code	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot
Q-waves	1:1	1	2		3	1	1		2					1	1		2
	2	3	1		4					1			1	1		1	2
	3						4		6	2			2		3		3
Axi d viation left	2:1		4		4	[illegible]	[illegible]		4		1		1	1	4		5
High ampl R wave left type	3:1		2		2	1	[illegible]		3	1	1		2	2	2		4
	3	1	3	2	6		3		3	1			1	3	1		4
ST depressi n	4:1																
	2	1			1	3			3					2	2		4
	3	2	3	2	7	1	4		5	1	4		5	4	7	1	1[illegible]
	4+5		7		7	3	4		7	4	1		5	5	10	1	16
	6+7		1		1	1			1								
T wav > 5 mm	5:1																
1 to 5 mm	2						1		1						1		1
Flat or diphasic	3	1			1	2	1		3					1	3		4
Low amplitude	4	3	16	3	22	13	21		34	5	3		8	12	21	2	35
LBBB	7:1						1		1	1			1		1		1
Ectopic beats v ntric	10:1-5	1		1	[illegible]	1	3		4		1		1	1	5	1	7
suprav ntric	6 8			1	1										1	1	2
Digitali therapy		2	2		4	3	2		5	2			2	3	1		4
Total examined		10	25	5	40	25	42	1	68	8	9		17	30	48	2	80

For Q wave and ST d pr ion ee appendix

Tabl 10 Di tributi n f th mo t pron un ed 1 tro ardi graphi chang during r aft r x rci e among re pond nt o twins a ording t au f d ath of index twin

		Mal								Female							
		IHD				not IHD				IHD				n t IHD			
	FCG de	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot
ST d pr i n	4:1	2	4		6	3	1		4		2		2	[illegible]	[illegible]		4
		3	6	1	10	3	8		11	3	3		6	11	13	1	25
	3		4	2	8	3	10		13	3	3		6	7	16		23
	4+5		4		4	2	4	1	7		1		1	4	4		8
	6+7		2	1	3	3	6		9					3	3		6
T wave																	
≥ 5 mm	5:1	1			1										1		1
-1 to 5 mm	[illegible]	1	1	1	3	1	1		[illegible]								
Flat r diphasi	3	1	2		3	1	1		2	2			2	5	4		9
Low amplitud	4	3	1	[illegible]	17	11	27	1	39	3	5		8	15	25		40
E t pi beat																	
freq ventric	10:1 4			1	1				[illegible]	1			1	1	6	1	8
oa ion ventric	5	4	4	1	9	4	5		9	1	3		4	3	9		12
supraventri	6-8		2		2	1	3		4		[illegible]		2	2	5		7
Total number		8	3	4	35	19	39	1	59	6	9		15	27	45	1	73

For Q wav s and ST d pr ssions app ndix

Table 11 Di tributi n of respond nt co twins not p rforming the ex rcise test according to r ason for non p rf rmance

	Males								Females							
	IHD				not IHD				IHD				not IHD			
	MX	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot
Statu po t myo ardial infar ti n		1		1									1			1
Cardia in uffi iency														1		1
Pa emaker			1	1												
Parkinson di ea e					1			1								
Statu post poliomyelitis															1	1
Rectal an er					1			1								
Orth paedic r a ons					1			1						[illegible]		2
Refusal						1		1								
Total numb r		1	1	2	3	1		4					1	3	1	5

T bl 1 Di tributi n of means f r V_{max} and final h rt rat a rding t au f d ath of ind x twin (ubj t n digit li th rapy omitt d)

	Male								F mal							
	IHD				not IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SD)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
V_{max} (kpm/min)	647 (91)	6 1 (37)	675 (95)	633 (33)	636 (43)	692 (30)	150 (0)	664 (6)	404 (6)	4 8 (49)		418 (37)	432 (.1)	127 (16)	400 (0)	4 8 (13)
Final h art rat (b at /min)	158 (5 0)	148 (3 7)	158 (7 8)	132 (2 9)	150 (4)	157 (2 9)	146 (0 0)	155 (. 4)	149 (11 7)	153 (9)		15. (5 3)	158 (2 3)	153 (7)	118 (0 0)	155 (. 0)
T tal numb r	8	3	4	35	19	39	1	59	6	9		15	7	45	1	73

– Tabl 13 Di tributi n f means f r h art v lume among re p nd nt co-twin acc rding to aus of death of ind x twin

	Mal s								Femal s							
	IHD				not IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	T t M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
H art volume (c /m BSA)	449 (25)	384 (13)	462 (33)	410 (12)	413 (16)	407 (13)	520 (0)	411 (10)	430 (.2)	397 (31)		412 (19)	368 (11)	353 (9)	400 (60)	360 (7)
Total numb r	10	5	5	40	5	41	1	67	8	9		17	30	45	.	77

BSA=Body surface ar a

Comment

ST-T change have been omitted from the tables in the ca e of two anemic co-twins with a hemoglobin concentration below 10 g % One of them a female DZ co-twin had be n treated at hospital for hyp r thyroidism and renal insuffi iency and had a serum creatinin lev l of 3 6 mg % H r partner had died from uraemia and the autopsy howed polycystic kidn ys Th urviving co-twin has possibly also poly cystic kidneys The other a male MZ co twin who e brother had committ suicide had signs of left ventricular hypertrophy with high amplitude R wave (3:1) and ventricular activation time >0 05 seconds He als had an enlarged heart at X ray with a relative size of 600 ml per square metre body urface He was diagno d as having a suspect cardiomyopathy These two subj cts sh w d no clinical signs of IHD and their ST depressions (4:1 2) w re not consid red indicative of IHD

Two of th co twin displayed physical and phono-cardiographic signs of congenital h art disea e and valvular heart di ease re pec tiv ly One of them a male MZ co twin whose partner had died from asthma bronchiale had dyspnoe on effort H was treated with digitalis and the ECG how d intraventricular block The probable diagnosis was atrial s ptal defe t The other co-twin a female DZ whose partner had died from malignant tumor of the breast reveal d no symptoms of ardia insuffici ncy The ECG was normal Th probabl diagnosis in thi ca e was mitral insufficiency

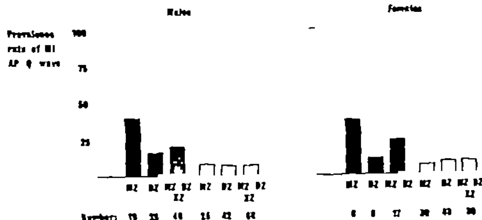
Grading of IHD

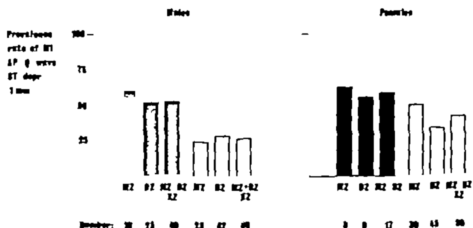
The umulative di tribution of differ nt manifestations of IHD is given in Table 14 Myocardial infarction verified at ho pital ha been taken a the harde t criterion of IHD Angina p tori and various ECG criteria are then ucc ssiv ly add d to myocardial infarction Figur 7 how the pr val n e of some of the e combinations The surviving co twin who e partn r died from IHD di play a consi t ntly high r prevalen e of th various IHD manifestations than tho who partn r died from other cau e than IHD Thi applie to both th male and the f male groups but i s m what more pronoun ed for th male parti ularly when th a se sment of IHD has be n ext nded t include ST d pres ions The diff rence between th male co-twins (MZ + DZ + XZ) whose partner did or did not die from IHD is t ti tic ally signifi ant (p< 0 05) not only wh n ST depr s i n >1 0 mm re includ d but also for ST d pre ions >0 5 mm Comparing th prevalence of the variou IHD manife tation within th ygo ity group it i the MZ co twin wh partn rs died from IHD who are consist ntly the

Tabl 14 Cumulativ di tributi n of diff rent IHD manif tati ns among r pond nt twins a ording to c us of d ath of ind x twin (odd numb r within par nth)

	Mal								Female							
	IHD				not IHD				IHD				not IHD			
	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot	MZ	DZ	XZ	Tot
Myoc rdi l infarcti n	3	3		6	1	1		2						1		1
+ Angina pectori	3	4		7	1			3	2	1		3		4		4
+ Q-wav (1:1 2)	4	4		8	2	3		5	3	1		4	2	4	1	7
+ ST d pr ion (4:1 2) during/ ft r ex rci	7	13	1	⁻1	6	12		18	5	5		10	15	16	?	33
+ T wav (5:1 2) during/aft r x rci	7	13	1	1	6	12		18	5	5		10	15	16	2	33
+ Q wave (1:3)	7	13	1	21	7	15		22	6	5		11	15	17	2	34
+ ST d pr ion (4:3)	9	17	3	29	9	23		32	8	8		16	⁻1	32	2	55
+ T wave (5:3)	9	17	3	29	9	3		32	8	8		16	21	32	2	55
+ LBBB (7:1)	9	17	3	29	9	23		32	8	8		16	⁻1	32	2	55
Total number	10	25	5	40	-5	42	1	68	8	9		17	30	48	2	80

For Q wave and ST d pres ions ee app ndix





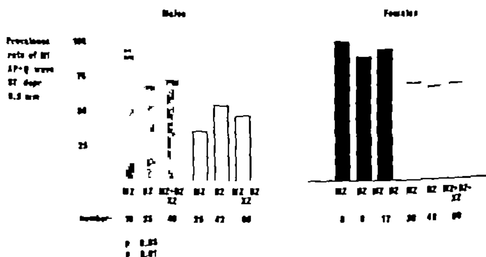


Fig 7 Prevalen e rate of some IHD manifestations among respond nt co twins Dark olumns indi at that th partner died from IHD and white column that partner died from other cause

most aff ted group They have signifi antly higher pr valence rates of IHD manife tation than their counterpart who e partn rs did not die from IHD The difference i significant at th 5 % level when ST depre ions ≥1 0 mm ar in luded and at th 1 % level when ST depressions ≥0 5 mm are in luded The female co twins eem to be proporti nally more affected than th males wh n ofter IHD criteria are in luded irrespectiv of th cause of death (IHD/not IHD) The differ nc between the pooled female zygo ity group (IHD vs not IHD) i al o ignifi ant ($p < 0\ 05$) when ST d pre ions ≥0 5 mm are included

Comment

The clinical ntiti myo ardial infarction angina p ctori and sudden d ath are usually includ d in the d finition of IHD Mo t epidemiologi al tudi s al o includ variou ECG finding in order to detect preclinical cases of IHD

Conc rning Q wave Q_1 i n arly always sugge tive of old infarction Th sp cificity of Q_{1-2} a an indi ator of IHD ha b en validated by aut psy and found to b high (Bjurulf t al 1967) All th pr nt co twins with myocardial infarction had Q_1 or Q_2 In sev ral population tudi Q_{1-2} (Ep tein t al 1965; Ro e 1971; Tibblin & Wilhelmsen 1971; B ngt on 19 3) and ometime $Q_{1\ 3}$ (Punsar & Karvonen 1973) have be n tak n a evid nc of the pr bable pr en e of IHD However in a fiv y ar follow up of n arly 13 000 men from even ountri s the pr en e of a Q_3 had no ignificant pr gno tic pow r for future IHD (Blackburn t al 1970)

ECG changes mainly ST hange in conn ti n with ex rcise are consid red to b u eful f r det cting pr clinical forms of IHD It must b remembered how v r that ome subj t with clinically documented IHD di play no ECG abnormality at ex rci e ju t as th re are other with abnormal ECG re pon e at x rci and n rmal oronary ngiography (Redwood & Epst in 1972) ST gm ntal d pr ion in respons t xerci ha b en hown t b a good predi tor of overt IHD (Mattingly 1962; Å trand & Lundman 1968; Bla kburn et al 1970; Doyle & Kin h 1970) Th ord r of ST gmental d pre sion is also dire tly d pend nt on th r lative load at xerci e (Blomqui t 1965) A study by Doan et al (1965) h wed that ompared to Ma t r two tep te t a n ar maximal te t in rea ed th e n itivity nin time in d t ting i chaemi ST d pres ions

ST d pre sions em to b a l p cific ign of IHD in women (Biörck 1946; Lepe hkin 1958; Åstrand 1965) Åstrand noted that th fr qu ncy of ST d pressions ≥0 5 mm among men hould be about

20 % at 55 years of age and about 35 % at 60 For females the expec frequency should be about 50 % at 55 years and over These figures a based upon submaximal work load with fixed target pulse for different age-groups (Åstrand et al 1967) In the present study too ST depre ions were more common among the women

When ST depressions were includ d in th criteria ugg ting IHD, the surviving male co-twins with partn rs who di d from IHD displayed these signs of IHD significantly more often than tho e whose partners died from other causes Th findings go in the same dire tion as tho e of Liljefors (1970) for 91 male twin pairs An exten ion of the ECG criteria of IHD to ST depressions ≥0 5 mm among f males also significantly ($p < 0\ 05$) discriminates co twin who e partn rs died from IHD from tho e whose partners died from other cau s In some studie (Epstein et al 1965; B ngtsson 1973) ST d pressions have not been coded in th presen e of high amplitude R wav (3:1) because in such ases ST depressi n were consid r d to reflect a hypertensive disease If this procedur had been adopted in thi study the signifi cant difference ($p < 0\ 05$) in IHD manifestation between the surviv female co twins w uld have be n non- ignificant but the significance recorded b t en th male co-twin would have been unchanged However experi nc fr m th Framingham study has shown that left ventricular hypertrophy (including ST depression and T-wave inversion) predicts th IHD incid n e over and above what could be accounted for by the on urr nt hyp rten ion (Kannel et al 1970; Dawber & Kannel 1972) Th y consider d th e abnormalitie to reflect not only a hypertensive hypertrophy but also IHD

The differ n s recorded in IHD manifestations certainly reflect a g n ti influ n e which is furthermore und rlined by th triking difference in IHD manifestations in spite of a reversed age factor b tw en the male MZ co twins who e partners did or did not die from IHD Th pos ible role of environmental factors is discus ed in some of the subs qu nt chapt r

2 BIOMETRIC FACTORS ASSOCIATED WITH ISCHAEMIC HEART DISEASE AMONG THE SURVIVING CO-TWINS

ANTHROPOMETRIC VARIABLES

Obe ity is usually defined in terms of weight and then compared to tandard or norms for th entire population or expre ed as relative weight Another mea ure of besity i kin fold thickn ss

Obe ity i gen rally considered to involv a sub tantial risk of IHD though th r lationship i om what uncl ar In the T cumseh tudy the prevalence of IHD was indep nd ntly correlat d with over-weight among men but n t among women (Ep t in et al 1965) The Framingham study has shown an as ociation b twe n ov rw ight and an increased incidence of angina pect ri and sudd n death but not myo ardial infarcti n (Kannel t al 1967) Multivariate analysi of the data from the ven ountry study (Key et al 1972) showed that n measure of relativ weight or obesity made a ignificant contribu-tion to future IHD wh n the fa t r of age blood pr ure erum ch l terol and smoking w re comparable

Gertl r & Whit (1954) f und no differen in weight between controls and men und r 40 with previou myocardial infarction The me omorphou body build was h wever mor common among th infarction group than among th o ntrols For sman & Lindegård (1958) studi d a imilar mat rial of p st infarction men under 56 They distinguished between two sub group in the mat rial n with above av rag l ngth w ight and turdin s fa t r and th other with small such factors In an autopsy tudy by Bjurulf (1959) on 110 patient aged 25-88 19 of whom had myocardial infarcti n th v rity of oronary athero-l ro i wa c rrelat d to th ize of the subcutan ous fat c lls but not to th ir number and al o to th mus le mas It was concluded that th grad of coronary ath ro clero i was more dependent on nvironmental influen than on genetic dispo ition Th result from a tudy by Björntorp & Sjö tröm (1971) al sugge t two forms of obe ity On i charact rized by a hyp rtrophy of fat ells and i of a mod rate d gr Thi type of ob ity has been hown to be as ociated with metaboli di turbance The oth r form has an increased number f fat c ll and i a iated with mu h mor ever ob ity In a ompari n b tw n po t infarction male and ontrol B rcht ld et al (1972) found n differenc with r gard to body fat and fat cell i In a tudy n women Bengtsson (1973) r ported no significant

difference with regard to overweight between control groups and one with myocardial infarction angina p otori and ECG signs suggesti of IHD respe tively In another study (Cramér et al 1966) on 173 males and 51 femal no correlation was found between coronary and graphic findings and relative weight

It mu t b born in mind however that obese persons are more likely to be hypert nsi e and hyperlipidaemic hyperglycaemic and hyperuricaemic and that this probably reflects chronic caloric imbalan e in susceptible peopl (Stamler 1973)

Material and M thod : ee Chapters I and II

Re ults

The distribution of the m ans of ome of the anthropometri parameter is shown in Table 15 There are no sub tantial differences among the males Th MZ o twin whose partners died from IHD ar omewhat heavier and hav a bigger arm circumference as well as more skinfold fat in th tric p ar a than either the DZ co-twins with IHD partners or the MZ o twins wh e partn rs died of oth r causes than IHD Thes differenc are not stati tically significant The female co-twins especially the MZ group whos partn r died from IHD are h avier and ha mewhat more ki ifold fat than the co twin whose partners died from other aus s than IHD but th e differ n es ar not statisticall signifi ant Th di tribution of overweight co-twin d fined a relative w ight ≥110 according to the earlier described weight/height index is hown in Table 16 Of the male co-twins wh se partners died fr m IHD 18 % are overweight compared to 24 % of those whos partner died from oth r caus s The corresponding figures among the f mal ar 53 % and 46 % None of the differ nces with regard to the oc urr n of ov rw ight is tati tically ignificant

C mm nts

W ight a d keletal mea urem nts were found to b chiefly dep ndent on g n ti fa to in twin tudie by O born & de G orge (1959) and Takkun n (1964) On the oth r hand Lundman (1966) and Lilj fors (19 in th ir t in tudi f und weight and skinfold thicknes to be dep nd nt n b th g n ti and nvironmental factor The o-twins who partner died fr m IHD d play d a tend ncy to gr at r r lativ weight and kinf ld fat mpared to tho e who e partn r died from oth r ca This was m marked among th f male e p cially the MZ co twins Th finding could indi at that thes p ramet rs in ome m a ure are lin to the development of IHD in f male

t bl 15 [i t ibuti n f me tor m anthropometri paramet r of re pond nt o twi s rding to
s of d ath f ind x twin

	Mal								Femal							
	IHD				n t IHD				IHD				not IHD			
	MZ M (I)	DZ M (SF)	XZ M (SF)	T t M (SF)	MZ M (SF)	DZ M (SF)	XZ M (SE)	Tot M (SF)	MZ M (SE)	DZ M (SF)	XZ M (SF)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
A	173 7 (1 4)	173 1 (0 9)	17 8 (1 8)	173 (0 7)	173 (1 1)	171 0 (1 1)	170 0 0	171 8 (0 8)	156 9 (-)	163 8 (1 5)		160 5 (1 5)	158 7 (1 1)	159 5 (0 9)	15 0 (2 0)	159 1 (0 7)
B	75 6 (3 1)	7 (1 8)	(0)	73 1 (1 4)	1 9 (2 4)	7 1 (1 5)	64 0 0	71 9 (1 3)	70 1 (7)	71 3 (4 6)	-	70 8 (4 0)	6 0 (1 5)	66 1 (1 9)	76 0 (6 0)	64 8 (1 3)
C	11 4 (1 8)	9 6 (0 6)	9 0 (1 4)	10 0 (0 6)	9 0 (0 7)	9 8 (0 4)	10 0 0	9 5 (0 4)	3 1 (1 9)	0 0 (0)	-	1 4 (1 4)	19 4 (1 0)	19 3 (0 7)	20 3 (3 3)	19 3 (0 5)
D	15 9 (1 6)	15 6 (1 1)	15 8 (1 1)	15 7 (0 8)	15 9 (1 3)	16 3 (1 0)	13 0	16 1 (0 7)	4 0 (3 4)	1 4 (2 8)		- 6 (1)	18 4 (1 3)	0 8 (1 2)	31 6 (6)	0 (0 9)
E	31 4 (1 0)	30 0 (0 5)	30 1 (0 3)	30 4 (0 4)	30 0 (0)	30 4 (0 4)	8 0 0	30 (0 3)	3 0 (1 9)	31 7 (1 3)		31 8 (1 1)	9 8 (0 6)	30 3 (0 8)	33 3 (0 3)	30 (0)
F	103 (4 3)	99 (4)	100 (2 5)	100 (1 9)	98 (3)	101 (1 7)	91 0	100 (1 6)	1 (8 3)	11 (7 3)		117 (5)	107 (3 3)	111 (8)	139 (16 0)	110 ( )
Tot	10	5	5	40	5	4	1	68	8	9		17	30	48		80

A=H ight m) B W ight kg; C=Skinf ld trio p area mm; D=Skinf ld ubscapular area mm; E=Arm circum f r nce cm; F=Relative weight

Table 16 Distribution of respondent co-twins with overweight (relative weight ≥110) according to cause of d ath of ind x twin

	IHD				not IHD			
	MZ No %	DZ No %	XZ No %	Tot No %	MZ No %	DZ No %	XZ No %	T t No %
Overweight male (relative weight ≥110)	3(30)	4(16)	0	7(18)	7(28)	9(21)	0	16(24)
Total number	10	25	5	40	25	42	1	68
Overweight female (relativ weight ≥110)	4(50)	5(56)	-	9(53)	11(37)	24(50)	2(100)	37(46)
Total numb r	8	9	-	17	30	48	2	80

BLOOD PRESSURE

El v t d l d p s ure ha been designated one of the major risk fa t r r matur IHD (Atherosclerosis Study Group 1970; Sisborg 19); t mler & Ep tein 1972; Stamler 1973) Data from 14 years f ll up of 5 127 m n and women in the Framingham tudy (Kannel et l 1971) hav hown that the risk of developing IHD i imply propor t nal to blo d pressure from th lowest to the highest l v l f r both m n and w men Furtherm re similar gradients of ri k were ob erved wh ther p r ons w re cla sified by their systolic or dia tolic pressure although the yst lic pressure displayed a strong r associati n with th ri k of d v loping IHD

A cording to Epstein (1964) familial aggregations of IHD eem to be c ndition d to om extent by hypertension which could thu be one of th mechanisms of gen tic transmission A genetic influence has been demonstrated f r e ssential hypertension in family tudie and studie on twins extensiv ly r viewed by Miall (1971) who supports th hypoth is that arterial pre sures are determined polygenically He also not s that familial fa tors a count for only one third of the variance of y tolic pres ur and one fifth of that of dia toli and that non-familial nvironm ntal factors presumably account f r the remaind r

Some of th twin tudi by O b rne et al (1963) Downie et al (1969) and Lilj f rs (1970) have hown blood pre ure to be pre-

dominantly under environmental influence as elucidated from comparisons between MZ and DZ intra pair variances whereas studies by Takkunen (1964) and Lundman (1966) have pointed to a stronger genetic influence Gen tic factor also eem to play a more important role in females to judge from the studies by Lundman and Osborne et al

Material and Methods: see Chapters I and II

Results

The di tributi n of means for asual and ba al blood pr ssur and pulse rates is shown in Tables 17 and 18 Casual and ba al systolic pre sures as well as diastolic phase 4 and 5 pressures are on av rage higher for the o-twins with partner who died from IHD than for tho whose partners died from other ause The difference are relativ ly marked among th females especially for casual systolic pre sure where the diff rence i significant ($p < 0.05$) Comparisons by zygosity show that the male MZ co twins whose partners died from IHD di play a slight tendency to have higher measured pr ssures than th DZ co twin in this IHD group For the femal s there i the rev rse tendency for casual pressur s the DZ co twins having th highe t value The casual syst lic pressure are also ignificantly ($p < 0.05$) higher for the femal DZ o-twins who e partners died from IHD than th female DZ co-twins who partners died from other caus than IHD The distribution of co twins with elevated blood pressur d fined here as basal systoli ≥ 160 mm Hg and/or ≥95 mm Hg diastolic pha e 4 is presented in Table 19 The occurrence of "hypertension a cording to this definition i 48 % among the mal o twins with partners who di d from IHD and 41 % among tho e whose partners di d from other aus th corresponding figures for th females being 59 % and 41 % None of the differenc s is statistically ignificant

Comment

Population studi s concerning blood pressure extensively reviewed by Tibblin (1967) how that blood pr ssure ri s with age that the systolic pressure increases more than the diastolic after 50 and that blood pre sur in old women i higher than in old men In the present inve tigation the blood pre sure spe ially the casual sy tolic are surprisingly high in the female who e partners had died from IHD To some xt nt thi could po sibly b explained by th fact that they were n averag 4 year old r as well as heavier than the female co twins wh e partner had died from other auses than IHD It has been found

Table 17 Di tribution of mean for n unl bl od pressure (B p) and pul e rate in respondent co-twins according to cause of d ath of index-twin

	Males								Females							
	IHD				not IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
A	167 5 (10 9)	166 0 (5 9)	147 0 (6 8)	164 0 (4 [illegible])	163 0 (5 5)	157 6 (3 4)	170 0 0	159 8 (9)	[illegible]	[illegible]		195 6 (6 8)	175 7 (7 1)	172 1 (4 9)	19[illegible] 5 (2 5)	174 0 (4 0)
B	99 5 (4 4)	96 0 ( )	88 0 (4 6)	95 9 (1 9)	93 0 (2 3)	93 0 (1 5)	95 0 0	93 0 (1)	[illegible] 1 (1 6)	100 [illegible] (1 8)	-	99 1 ([illegible])	94 0 (8)	93 5 ([illegible] 3)	112 5 (12 5)	94 2 (1 8)
C	98 0 (4 [illegible])	95 4 (3)	88 0 (4 6)	95 1 (1 9)	91 6 ([illegible] 4)	9[illegible] 1 (1 5)	95 0 0	9[illegible] 0 (1 3)	97 5 (4 4)	100 0 (3 8)	-	98 8 (8)	9[illegible] 0 (8)	91 7 (4)	112 5 (1[illegible] 5)	92 3 (1 8)
D	81 4 (4 5)	76 5 (2 9)	66 0 (3 8)	76 4 ([illegible] 3)	75 6 (2 9)	77 8 (2 3)	68 0 0	76 9 (1 8)	78 3 (5 3)	78 0 (4 8)	-	78 1 (3 4)	83 9 (3 3)	83 4 (2 4)	79 0 (19 0)	83 5 (1 9)
Tot	10	5	5	40	[illegible]5	4[illegible]	1	68	8	9	-	17	30	48	2	80

A=Systolic B p ; B=Diastolic B p phase 4; C=Diastolic B p phase 5; D=Pulse rate beats/min

Tabl 18 Di tribution f m ans for ba al blood pr ur (B p) and pul rat in r pond nt o twins rding t au of d ath of ind x twin

	Mal								F mal							
	IHD				n t IHD				IHD				n t IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	T t M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	T t M (SE)	MZ M (SE)	DZ M (SD)	XZ M (SE)	Tot M (SE)
A	154 5 (11 6)	147 (5 0)	137 0 (7 5)	147 8 (4 3)	147 0 (4 7)	140 5 (2 6)	150 0 0	143 0 (2 4)	172 5 (13 2)	166 1 (7 3)		169 1 (7 1)	152 3 (5 7)	154 4 (4 7)	175 0 (10 0)	154 1 (3 5)
B	97 0 (5)	91 4 (1 6)	85 0 (5 7)	9 0 (1 8)	90 (2 6)	88 8 (1 3)	95 0 0	89 4 (1)	94 4 (4)	89 4 (3 7)	-	91 8 (2 7)	86 8 (2 5)	88 1 (2 1)	11 5 (12 5)	88 3 (1 6)
C	95 0 (4 6)	90 8 (1 7)	85 0 (5 7)	91 1 (1 7)	89 6 (2 6)	87 5 (1 5)	95 0 0	88 4 (1 3)	93 8 (4 2)	88 9 (3 8)	-	91 2 (2 8)	85 7 (6)	87 0 (- 1)	110 0 (10 0)	87 1 (1 6)
D	75 0 (5 5)	71 6 (3)	62 8 (4)	71 5 (1 8)	71 8 (5)	74 2 (2 3)	68 0 0	73 - (1 7)	70 8 (4 1)	68 2 (3 6)	-	69 4 (6)	77 4 (2 9)	76 5 (1 9)	72 0 (8 0)	76 7 (1 6)
Tot	10	25	5	40	25	42	1	68	8	9		17	30	48	[illegible]	80

A=Syst lic B p ; B Dia tolic B p phase 4; C=Dia tolic B p phase 5; D=Pulse rate beats/min

Table 19 Distribution of respondent co-twins with elevated ba al blood pressure (≥160 mm Hg systolic and/or ≥95 mm Hg dia tolic phase 4)

Elevated blood pres-sure	IHD MZ No %	IHD DZ No %	IHD XZ No %	IHD Tot No %	not IHD MZ No %	not IHD DZ No %	not IHD XZ No %	not IHD T t No %
Males	6(60)	12(48)	1(.0)	19(48)	12(48)	15(37)	1(100)	28(41)
Tot	10	25	5	40	25	42	1	68
Females	5(63)	5(56)	-	10(59)	16(53)	15(31)	2(100)	33(41)
Tot	8	9		17	30	48	2	80

(Humerfeldt 1963) that wh n age is held constant systolic blood pressure increas only about 3 mm Hg for each 10 kg incr a e in weight and dia toli blood pre ure only 2 mm Hg The twin studie m ntion d arl r sugg t that the genetic influence on blood pre sure may increa o with age and may b greater in women The pres nt co-twin who e partn r died from IHD di play a slight tendency to higher blood pr ure and th high t value occur in the MZ co-twins exc pt in the ca of a ual y t li and dia tolic pre sures in the femal s where the high t alu s m from the DZ co twins ECG signs indicative of IHD w r f und by B ngts n (1973) to be fairly unreliabl in this re p ct and it wa on id red that in women they ould probably be cau d by hyp rt nsi n It is thus conceivable that the relativ ly high blood pre ures re ord d in the female co-twins whose partners di d from IHD are partly re ponsible for th high pr valence of ECG igns sugge tiv of IHD

LIPIDS

Ch lesterol like hyp rt nsion has be n d ignated on of the maj r ri k factor for premature IHD (Athero clero i Study Group 1970; Simborg 1970; Stamler & Ep tein 1972; Stamler 1973) The average serum hole terol lev l f per on with myocardial infarcti n is higher than in the normal p pulation (Biörck et al 1957; Carl on 1960; Gu tafsson et al 1972) and ev ral prospective tudi s have demonstrated str ng statisti al associations betw en elevated serum cholest rol and IHD in m n (Key et al 1970; Ro enman et al 1970; Kann l et al 1971; We tlund & Nicolay n 197.) The Framingham study has shown that el vat d erum hole terol in women under 50 is associ

with an increased risk of developing IHD (Kannel et al 1971) Elevated holest rol has also b en found in women with manifest IHD (Mulcahy et al 1967)

Fewer r ports have be n publish d on erum triglycerides and IHD Prospective studi s have shown that elevated triglyceride are likewi a ociat d with an increased ri k of myo ardial infarction (Carlson & Böttiger 1972; Tibblin 1972) Significantly higher serum triglycerid were found by Bengt on (1973) in women who had had myocardial infarction and in women with ECG chang sugge tive of IHD a ompared to th g n ral female population No ignificant ov r-representation of high serum triglyceride wa f und in women with angina pectori

Lipoprot ins can b clas ified (Beaumont et al 1970) by means of ultra entrifugation (Gofman et al 1954) or el ctrophoresis Fredrickson & Le 1965) Applying their lipoprot in ph notyping system to a larg numb r of pers ns with a primary in rease in plasma lipids Fredrickson and co-workers managed to di tinguish betw en five primary hyperlipoproteinа mias Three of them (II-IV) are associated with an increased risk of IHD Of th e tablished hyperlipoproteinaemia the clas ical familial hyperchol terolaemia (type II a) is thought to be inherited by an aut somal dominant g n with a high pen trance; it can occur in a homozygoti form (Fredri kson 1971; Leading article Lancet 1971) The primary familial hyperlipoproteinaemias however usually include only the gros hyperlipidaemic ca es which are fairly uncommon in the general populati n In a recent study on 412 fir t degre relatives of 101 young survivors of myocardial infarction (Nikkilä & Aro 1973; Aro 1973) th mean lev ls of s rum cholesterol and erum triglycerides were ignificantly high r than in a control population but lower than in th ind x patients Hyp rchole terolaemia (type II a) occurred 1 8 times hypertriglyc ridaemia (type IV) 1 3 times and combined hyperlipidaemia (type II b) 2 5 tim more frequently in relatives than in ontrols It was furthermore concluded that one-third of patients with prematur IHD hav a familial trait of hyperlipidaemia but it could not b tabli hed whether this trait i inherited or produced by environmental fa tors

Some of th earlier twin studies have shown that the variability f rum chole terol i du to both environmental and genetic factors (Osborne et al 1959; Mc Donough et al 1962; Meyer 1962; Jensen et al 1965; Rifkind et al 1968) Compari ons between twins living together and apart howed a mall r intra-pair variance among the former indi ating an influence from the environment But as the intra-pair

difference was significantly greater for the DZ than the MZ pairs there seems to be a genetic influence too

However the study by Gedda & Poggi (1960) on twin pairs below the age of 20 show d that genetic factors strongly influenced th variabili ty of cholesterol In his study on 196 smoking discordant male and female twin pair using th Swedish Twin Registry Lundman (1966) found cholesterol to be under both environmental and genetic influence while for triglyc rides and phospholipids the genetic component was more obvious in the female pairs Studying 91 mal twin pairs Liljefors (1970) found ɪdence of a genetic influ nce on the cholesterol level from the ıgni ̂ cantly maller variance in the MZ than the DZ twins There was no u h evidence of heredity in respect of tri glycerides

Material and Meth : s e Chapters I and II

Re ult

The means ̂ ̅ ol ̅ r l and triglycerides are given in Tabl 20 Becau e th t i eiide values showed a skewed distribution th y ere tran f r n h i̅ logarithms before making stati tical comparisons Both th hol e l and the triglyceride values are higher on average for th mal twins whose partners di d from IHD than for th se wh p tn r d ed fr m other cause The difference is not statis ti ally ignif ant The highest lipid value among the male co-twins ar tho e of the DZ o-twins whose partner died from IHD Among the f male co twins ther is a imilar trend for chole terol with higher means for th co twins with partn rs who died from IHD The trend for triglyc ride ı the r vers of this mainly due to comparativ ly low valu among th DZ co twins whose partner died from IHD None f the differ nce within the variou male and female groups i statistically ignificant

The di tribution of co twins with elevated chole t rol (≥ 50 mg/ 100 ml) and/or elevat d triglyceride (≥150 mg/100 ml) is given in Table 21 Of the male co-twins who e partners died from IHD 60 % had elevat d ch lesterol and/or triglyceride value compared to 50 % of tho e whose partn rs died from oth r cau es Among the female co-twins the corresponding figure were 76 % vs 68 % None of the differences is statistically ignifi ant

Comm nts

Men and wom n oft n have much the ame holesterol level from 20-50

T bl 20 Di tributi n f mean f r chol t rol and triglyc rid in re pondent o twins a cording to aus of d ath f ind x twin

	Male								Female							
	IHD				not IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)
A	221 0 (8 4)	239 6 (11 8)	14 0 (20 0)	231 8 (8 1)	210 6 (9 2)	226 5 (8 0)	80 0 0	221 5 (6 1)	280 9 (35 2)	272 ˆ (21 8)	- -	276 3 (19 5)	269 0 (9 6)	243 5 (8 7)	307 5 (17 5)	254 7 (6 5)
B	127 5 (17 8)	167 8 (18 9)	151 0 (27 4)	155 6 (13 2)	144 8 (14 1)	137 4 (11 1)	110 0 0	139 7 (8 5)	168 1 (28 2)	117 8 (9 9)	-	141 5 (15 1)	150 5 (15 0)	157 7 (17 8)	175 0 (30 0)	155 4 (12 0)
C	06 (0 07)	2 17 (0 04)	14 (0 10)	2 14 (0 04)	2 1 (0 04)	2 09 (0 03)	2 04 0	ˆ 10 (0 02)	2 19 (0 07)	2 06 (0 05)	- -	ˆ 12 (0 04)	2 12 (0 04)	2 14 (0 03)	2 24 (0 08)	2 14 (0 02)
Tot	10	25	5	40	25	42	1	68	8	9	-	17	30	48	2	80

A=Chole t rol (mg/100 ml); B Triglyc ride (mg/100 ml); C=Log triglyceride (mg/100 ml)

difference was significantly greater for the DZ than the MZ pairs there seems to be a genetic influence too

However the study by Gedda & Poggi (1960) on twin pairs below th age of 20 showed that genetic factors strongly influenced the variabili ty of cholesterol In his study on 196 smoking discordant male and female twin pairs using the Swedish Twin Registry Lundman (1966) found cholesterol to be under both environmental and g netic influence while for triglycerid and phospholipids the gen tic component was more obvious in the female pairs Studying 91 male twin pairs Liljefor (1970) found evid nce of a genetic influence on the ch lesterol level from the signif cantly smaller variance in th MZ than the DZ twins There was no u h evid nce of heredity in respect of tri glycerides

Mat rial and M thod : se Chapters I and II

Result

The means for ole t rol and triglyc rides are given in Table 20 Because th tr gl cerid values showed a skewed di tribution they were transformed to th ir logarithm before making tatistical comparisons Both the hol t ol and the triglyceride values are higher on average for th male o twins wh s partners died from IHD than for tho e who e partner di d from oth r causes The difference is n t t tisti ally ignifi ant The highe t lipid values among the male o-twins ar th se of the DZ co-twins whose partners died from IHD Among the f mal co twins there is a imilar trend for cholesterol with higher means for the co twins with partners who died from IHD The trend for triglycerid s i th reverse of this mainly due to comparatively low valu among th DZ co twins whose partners died from IHD None of the differ nces within the various male and female group i statistically significant

The distributi n of co twins with elevated cholesterol (≥ 50 mg/ 100 ml) and/or elevated triglycerides (≥150 mg/100 ml) is giv n in Tabl 21 Of the male co twin who e partner died from IHD 60 % had elevated chol st rol and/or triglyceride values compared to 50 % of those whose partn rs died from other cause Among the female co-twins the corresponding figur were 76 % vs 68 % None of th differences is stati ti ally ignifi ant

Comments

Men and women often hav much the same chol sterol level from 40-50

years of ag but after that the values tend to be higher in female The triglycerid level is usually lower in f males than males It i als vell kn wn that lipid level increase with age (Beaumont et al 1970) In th U S A the average serum chol sterol l vel increases with age in b th men and women until about 40 y ar when it level ff in men but continues to rise in women until about 60 year of age (Simborg 1970) In view of these finding it is po ible that th diff renc in cholest rol valu s between the f male o-twins who e partner died or did not die from IHD is to s me xtent a r flection f the ag diff rence (4 years) The twin tudy by Lundman (1966) showed only a weak a ciation between ag and rum lipid l vel in mal but highly ignificant correlations in female p cially among the MZ twins; th subjects howev r were on av rage about 10 years younger than th twins in th present inve tigation Trigly erid showed th same trends as h lesterol am ng th mal co-twins but th r vers trend among the female How ver it mu t b born in mind that ome f the differ nc s could be due to random variation becau the group were r latively small esp cially the f male IHD group

Th cut off points u d here to denote levation of chole terol and/or trigly erid s have been cho n fairly lib rally It i however well kn wn that th re is no evid n of a criti al l vel for th e factors In th Po ling Project in th U S A men with a chol terol value over 250 mg/100 ml carried a 10 y ar IHD risk about twice as high a the remainder of th p pulation (Stamler & Ep tein 1972) Th St ckholm Pro p otiv Study (Carls n & Böttiger 1972) al o show a linear r lati n to ri k ov r the ntir range of triglyc rid and chol st rol n ntrations

DIABETES MELLITUS

The as ociation betw n diab t mellitu and IHD ha long b en rec g nized An increas d incidence of IHD i found not only in per ons with ov rt diabet s mellitus but al o in tho e with impaired glu o e tol ranc (O trand r et al 1965; Ep tein 1967) The Framingham data show a higher incidenc of IHD in diabeti women than in diab ti men (Kannel et al 1967) Conv r ely a high proportion of subj t with IHD v re found to hav diab te mellitus (Siever et al 1961 Si v r 1963; Wahlb rg 1966; Simborg 1970) Survivor of myocardial infarc tion had abnormal intravenous glu tol ran more oft n than c ntrol (Wahlberg 1966) Th re eems to be g n ral agreem nt on the h reditary

character of diabete mellitus (Gedda & Brenchi 1970) although this has been difficult to assess in population studies (Epstein 1964) However twin studies have shown considerably higher concordanc regarding diabetes mellitu in MZ pairs compared to DZ pairs (Then Berg 1938; von Verschuer 1958; Joslin et al 1959; Harvald & Hauge 1965) In a study on MZ twins discordant with respect to ov rt dia betes mellitus Cerasi & Luft (1967) found a closely imilar insulin response after glucose infusion indicating a genetic influ nce

Material and M thod : ee Chapters I and II

Results

The prevalence rates for diabetes mellitu diagno ed earlier and the combination of this and fasting blood ugar >100 mg/100 ml are bout the same for the male co-twins irrespective of the cau of d ath of the partner (IHD/not IHD) (Table 22) The prevalence of overt di bet mellitus is especially high among the MZ co-twins who partn rs had not died from IHD (20 %) and it differ significantly ($p < 0\ 05$) from the prevalence (0 %) among the DZ co twins in the same group However it is worth noting that the 5 MZ co-twins who e partn rs died from other cause than IHD who had diabetes mellitus were all concordant in this respect i e their d ea ed partner had al o had ov rt di betes m llitu but this was not the cause of death in any of thes

Table 22 Prevalence of diab tes mellitus and/or fasting bl od sugar >100 mg/100 ml in surviving co twins according to cause of death f index twin

	IHD								not IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	N	%
Males																
A	1	10 0	2	8 0		0 0	3	7 5	5	20 0		0 0	-	0 0	5	7 4
B	1	10 0	4	16 0		0 0	5	12 5	5	20 0	2	4 8	-	0 0	7	10 3
Tot	10		5		5		40		25		42		1		68	
F males																
A	1	12 5	1	11 1	-	0 0	2	11 8	2	6 7	4	8 3		0 0	6	7 5
B	2	25 0	1	11 1		0 0	3	17 6	3	10 0	4	8 3	-	0 0	7	8 8
Tot	8		9				17		30		48		2		80	

A=Diabete m llitus; B=Diab te mellitus and/or blood sugar >100 mg/100 ml

ca Th female co twins whose partner died from IHD have ome-what higher prevalence rates than the co twins who e partn rs died from other cau s Kone of the differences among the females is tati tically ignificant Diabetes mellitus wa th cause of d ath in two of th dec a ed twins in the present mat rial of death di cordant pairs One of their surviving co-twins (f male DZ) al o had ov rt diabete treated with tablets Her fa ting blood sugar wa 187 mg/100 ml Th other (male DZ) di play d no sign of diabetes mellitus having a fasting blood sugar of 81 mg/100 ml and no glu o - uria

Comments

In th pre nt inv tigation overt diab te mellitus and el vated fasting blood ugar do not app ar to be responsible for the ignifi-cantly higher prevalenc of IHD manif tations am ng the male co twins who partn r died from IHD compared to th e who partn r did not di from IHD be aus th preval nce of ov rt diab te mellitus and el vated fa ting blood ugar was about the am in both group K ith r did th twin tudy by Liljefor (1970) indi ate that th pre nc f diabet m llitu i a det rmining fa tor for th o cur r nc of IHD in twins who pr bably had IHD in the IHD di ordant pair However in oth r studi an overrepr entation of diabet mellitus ha been r port d am ng case with IHD (Siev r et al 1961; Siev rs 1963; Wahlberg 1966; Simborg 1970) That gen ti fa t r ar f importance for th o currence of diab te i underlined by the fa t that 5 male MZ pairs (not IHD death di rdant) w r o n ordant with r sp t to overt diab t mellitu Th preval n e of diabet mellitus als ms rath r high am ng the urviving c twins whether r not th ir partn r died from IHD ompared to th general population (Grönb rg et al 1967; B ngt n 1973)

URIC ACID

Clini al and pidemiologi tudie hav hown that hyp ruri aemia i mor ommon in IHD ubj t than in other (Gertl r & Whit 1954; Myer t al 1968) El vat d uri a id ha al o be n a iated with an increa ed future risk of dev loping IHD (Kann l et al 1967)

The po ibl tiologi rol of uri acid in th d vel pment of IHD i n t l ar Gertl r & Whit (1964) ugg ted that uri a id ould increa the adh iv n of lipid t art rial wall It ha al o

been proposed that uric acid influences thrombocyte aggregation (Newland 1968) Studies by Theorell (1971) have shown that uric acid production is high in persons who have had myocardial infarction and that after the infarction the subjects do not regulate their renal excretion of uric acid as efficiently as do healthy subjects

Twin studies have documented an influence of hereditary factors in the variation of uric acid levels (Harvald A Hauge 1955; Jensen et al 1965; Liljefors 1970)

Material and Methods: see Chapters I and II

Results

The mean value for uric acid in the surviving co-twins with and without diuretic treatment are presented in Table 23 Non of the differences betw en means among males and females re p ctively is statistically ignificant; this applies both to those with and to those without diuretics

The distribution of co twins with elevated uric acid (≥6 mg/100 ml) is given in Table 24 which includes tho e on diuretic therapy Of the male co-twins whose partn r died from IHD 50 % have elevated uric acid compared to 29 % of those who e partners died from other c use With the exception of one MZ co twin wh se partner had not died from IHD all the male co-twins on diuretic therapy have elevated uric acid Among the females the corre ponding figures are 18 % compared to 10 %; out of 18 f male co-twins on diuretic therapy 5 hav el vated uric acid (2 MZ with IHD partner and 3 DZ whose partners died from other causes) The difference in the o currence of elevated uric acid are not tati tically ignificant

Comments

As a larger prop rtion of the subjects whose partner had died from IHD were on diuretics the analyses of the result are of cours bia ed Wh n th co twins on diuretic th rapy are ex luded only slight difference are found among the others The female MZ co twins with partner who had died from IHD have high r uric acid lev ls on average than eith r th DZ twins in the same IHD group or the MZ twins who e partner had not died from IHD; to om extent thi may be due to difference in weight as there is known to be a p sitive correlation between uric acid and obe ity (Myers et al 1968)

Th differ nce in the occurrence of elevated uric acid are mainly due to the in lusion of p r ons re eiving diuretic treatment s that

Tabl 23 Di tributi n f mean for uri acid in re pond nt o-twin with and without diur tio tr at ment a rding t au f d ath f ind x-twin

	Male								Femal							
	IHD				n t IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XZ M (SE)	Tot M (SE)
No diuretio th rapy:																
Uri acid (mg/100 ml)	5 5 (0 2)	5 4 (0 3)	6 8 (0 7)	5 6 (0 2)	5 2 (0 3)	5 5 (0 2)	5 7 (0 0)	5 4 (0 1)	5 3 (0 3)	4 8 (0 2)		5 0 (0 2)	4 2 (0 2)	4 7 (0 2)	5 5 (0 0)	4 5 (0 1)
Number	7	23	5	35	-3	42	1	66	5	7		12	24	42	1	67
Diuretio th rapy:																
Urio acid (mg/100 ml)	6 4 (0 1)	6 9 (0 7)		6 6 (0 3)	4 6 (1 5)	-	-	4 6 (1 5)	7 4 (0 8)	4 8 (0 0)	-	6 3 (0 8)	4 8 (0 3)	6 0 (0 3)	6 0 (0 0)	5 5 (0 3)
Numb r	3	2		5					3	2	-	5	6	6	1	13

Table 24 Distribution of respondent co-twins with elevated uric acid (≥6 mg/100 ml) according to cause of death of index-twin

Elevated uric acid (≥6 mg/100 ml)	IHD MZ No (%)	IHD DZ No (%)	IHD XZ No (%)	IHD Tot No (%)	not IHD MZ No (%)	not IHD DZ No (%)	not IHD XZ No (%)	not IHD Tot No (%)
Males	5(50)	11(44)	4(80)	20(50)	8(32)	12(.9)	0	20(29)
Tot	10	25	5	40	25	42	1	68
Females	3(38)	0	-	3(18)	0	7(15)	1(50)	8(10)
Tot	8	9	-	17	30	48	2	80

in this study the etiologic role of uric acid is difficult to establish It seems reasonable to suppose however that an elevated uric acid level provoked by diuretic therapy could still act a a risk factor for IHD

HEMOGLOBIN HEMATOCRIT AND ERYTHROCYTE SEDIMENTATION RATE

Elevated hemoglobin values have been as o iated with an increa ed risk of IHD in men (Dawber & Kannel 1961; Böttiger & Carl on 1972) In a follow-up study of 50 year old men in Gothenburg (Tibblin 197?) a high hematocrit level was associated with the incid nce of both fatal and non fatal myocardial infarction but it also correlated with d ath from other causes than IHD esp cially canc r In the Sto kholm Pro pective Study an elevated erythrocyte s dimentation rate (E S R) has also been associated with an increased risk f IHD (Carl on & Böttiger 1972)

Material and Methods: se Chapters I and II

R sults

Table 25 give the mean for h moglobin hemat crit and erythrocyte sedim ntation rate (E S R) Both the male and the female valu s for hemoglobin and h matocrit are on averag omewhat low r for the o-twins whos partner di d from IHD than for thos whos partn rs died from oth r au es although the differ nces are n t tati tically significant With r gard to E S R the opposite applie The differenc between zygosity groups are also only light and non signifi cant

Tabl 5 Di tributi n f mean f r hemoglobin (Hb) h mato rit (H t) and rythro yte dimention rat (E S R) in r pond nt twin a ording to au e of d ath f ind x twin

	Mal								F male							
	IHD				n t IHD				IHD				not IHD			
	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)	MZ M (SE)	DZ M (SE)	XX M (SE)	Tot M (SE)
Hb (g/100 ml)	14 9 (0 4)	14 5 (0 3)	15 9 (0 3)	14 8 (0 2)	15 0 (0 3)	15 6 (0 3)	1)	15 4[1] (0 -)	14 3 (0 4)	13 5 (0)		13 8 (0 -)	14 0 (0)	13 9[1] (0 2)	8 8 (3 7)	13 8[1] (0 2)
Hot (%)	44 0 (1 0)	43 1 (0 9)	46 0 (0 9)	43 7 (0 6)	44 8 (0 8)	45 8 (0 7)	1)	45 4[1] (0 5)	41 8 (0 9)	40 - (0 9)	-	40 9 (0 7)	41 7 (0 6)	41 6[1] (0 7)	27 5 (12.5)	41 3[1] (0 6)
E S R (mm/hour)	16 8 (5 8)	15 4 (2 9)	6 2 (1)	14 6 (3)	11 7 (1 9)	11 5 (1 7)	6 0 (0 0)	11 5 (1 3)	20 9 (5 8)	18 6 (3 4)	-	19 7 (3 -)	16 5 (2 4)	17 8 (2 2)	26 0 (2 0)	17 5 (1 6)
T tal number	10	25	5	40	5	4-	1	68	8	9	-	17	30	48	2	80

1) Data missing from on subject

Comments

As already mentioned the Stockholm Prospective Study has id ntified high hemoglobin as a factor a sociated with an increased risk of IHD (Böttiger & Carlson 1972) But the same study has also demonstrated a positive correlation regardless of age between the plasma lipids cholesterol and triglycerides and hemoglobin (Böttiger & Carlson 1973) Hemoglobin as a risk factor for IHD might thus be explained not by the high hemoglobin value as such but by the concomitant increase in plasma lipids (Böttiger & Carlson 1973) Furth rmore the Stockholm Pro pectiv Study ha established a positive correla tion between elevated E S R and cholesterol as well as triglyc rides (Böttiger 1973) while a rec nt study on a ymptomatic hyperlipid aemic person showed significantly elevated E S R ompared to normo-lipidaemic controls (Böttiger et al 1973) Th authors suggested that a possible reason for the raised E S R could be that th hyper lipidaemia cau silent vascular disea e (diagnosed as ST depr s ions during exerci) which in turn produces an elevated E S R

3 ENVIRONMENTAL FACTORS ASSOCIATED WITH ISCHAEMIC HEART DISEASE AMONG THE DEATH DISCORDANT PAIRS

SMOKING

The relation of cigarette smoking to h alth has be n reviewed in reports from th Royal Colleg of Physicians in London (1971) and th Surgeon General of the U S Public H alth Servi e (1972) A causal a sociation probably exi ts between cigarette m king and lung an er a ell as chronic obstructive lung disease The question of a cau al association with IHD has been much debated From the studie reviewed in the above reports it i obvious that cigarette smoking i ass ciated tatistically with the exces male mortality from IHD The connection between igarette smoking and non-fatal myocardial infarcti n al o seems to be strong but for angina pectori it i more uncertain (Jenkins et al 1968; Seltzer 1968; Simborg 1970) In most tudies pipe and cigar smok r have not shown any increa ed ri k of IHD (Fletcher & Horn 1970) Although IHD death rate are much lower in women than in men an incr ased mortality from IHD ha been r ported for women smokers too (Hammond 1966)

Both acute and chronic effect of smoking on the h art have been discus ed Among th acute eff ct it ha b n propo ed that th effect of arbon monoxid or nicotine may aggravat myo ardial anoxemia when c ronary artery flow i already r duced and it i conc ivable that the risk of riou arrhythmia is enhan ed by nic tine via the relea of atech lamine (Fl t her & Horn 1970; Rose 1973) A chronic effect has be n e n n th relationship between ronary athero clero is and cigarette moking (Auerba h et al 1965) Howev r Dawber & Kannel (1972) from the Framingham study consider that th cigarette habit rather than tending t a celerate atherogen si triggers coronary attacks in per ons predi posed by a suffi i nt degre of coronary ath ro lerosi The r sult of that tudy hav shown that the risk of IHD i not as iated with th duration f the habit but only with th daily int nsity

Some pidemiol gi evidenc nflicts h wever with the overall pictur giv n by th major follow-up studi Th fir t 5 y ar follow up of nearly 13 000 men in even countrie (Key et al 1970) showed that smoking was r lated to later myocardial infarction and death from IHD in th U S A but n t in the oth r ountrie which in luded Ea t Finland with the high t male IHD mortality in the world

One of the arguments against the findings of conventional population studies is the fact that smokers are self-selected and that other factors may be involved in the causation of IHD

It has be n maintained by Friedman & Rosenman (1972) that persons with a particular behavior pattern type A characterized by aggressiveness striving and time c nsiousness more oft n develop myocardial infarction than the more placid type B; and the type A person is also more likely to be a heavy smoker

Qu tionnaire tudie on the Sw di h as well as the U S Twin Registries di closed an a sociation for males between smoking and angina pectoris when the results were analysed on a non pair basis but comparisons within smoking discordant pairs showed no associ tion with smoking for the MZ twins (report from sympo ium in San Juan Puerto Rico 1969) The e result are supported by a clinical study on 196 smoking di cordant pairs (Lundman 1966) from th Swedish Twin Regi try no difference being found with regard to IHD within the smoking discordant pairs

Material: see Chapt r I

Methods

Information about m king habits wa obtain d from qu stionnaire r plie rec ived in 1967/1970 If the twins had an wered in 1967 well a 1970 the latter report wa used

R ult

Smoking habit in the male and female death di cordant pairs a cording to the questionnaires ar hown in Tables 26-28 When pr s nt and former smoker ar combined (Table 28) the percentage distribution of male mok r i omewhat but not significantly higher f r the IHD c mpared t th not IHD death dis rdant pairs Of th mal twins who di d from IHD 83 % w re mokers as against 85 % of their surviving co twins while 76 % of th e who died from other caus than IHD were mokers compared with 72 % of their surviving co twins Th corre ponding figure for igarette smokers in the IHD d ath discordant pairs are 50 % and 55 % compar d to 40 % and 5- % among the not IHD death discordant pair Nor are the e diff ren es ignificant

There were mor form r igar tte smok rs (Table 26) among the decea ed male twin than th urviving co twins e pe ially among th se who died from IHD

With resp ct to zygo ity group (Table 28) 60 % (6 of 10) f th

Table 26 Smoking habit in male d ath di rdant pair a rding t earli r qu ti nnaire 1 r ntage di tribution

	IHD								not IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Non smok rs	10	20	20	16	20	0	18	15	20	16	16	19	0	0	24	18
Total present smokers	40	80	52	68	60	60	50	70	56	60	52	67	100	100	54	65
Total cigarett mok rs	30	80	24	24	60	60	30	43	12	32	26	36	100	100	22	35
1 10 cigar tt s daily	20	30	16	1	40	20	20	18	8	28	14	21	100	0	13	24
≥11 cigarettes daily	10	50	8	12	20	40	10	25	4	4	12	14	0	100	9	12
Cigar and/or pip	10	0	28	44	0	0	20	28	44	28	26	31	0	0	32	29
T tal f rmer smok r	50	0	28	16	0	40	33	15	24	24	21	14	0	0	22	18
Total cigarette smok rs	30	0	16	12	20	40	20	13	24	20	14	14	0	0	18	16
1 10 cigarette daily	10	0	16	8	20	20	15	8	16	16	10	14	0	0	12	15
≥11 cigar tt daily	0	0	0	4	0	20	5	5	8	4	5	0	0	0	6	1
Cigar and/or pipe	20	0	1[illegible]	4	0	0	13	3	0	4	7	0	0	0	4	1
Total number	10	10	25	25	5	5	40	40	5	25	42	42	1	1	68	68

D=Dec a ed twin; S=Surviving twin

Table 27 Smoking habits in female death discordant pairs according to earlier questionnaires Percentag distribution

	IHD								not IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Non smokers	75	50	78	67		-	76	59	70	77	85	79	50	100	79	79
Total pre ent smoker	25	13	11	33	-	-	18	24	30	23	15	17	50	0	21	19
Total cigarette smoker	25	13	11	33	-	-	18	24	27	23	15	17	50	0	20	19
1 10 cigarettes daily	13	13	11	11		-	12	12	20	23	10	10	50	0	15	15
≥11 cigarettes daily	13	0	0	22	-	-	6	12	7	0	4	6	0	0	5	4
Cigar and/or pipe	0	0	0	0	-		0	0	3	0	0	0	0	0	1	0
Total former smok rs	0	38	11	0		-	6	18	0	0	0	4	0	0	0	3
Total cigarette smoker	0	38	11	0	-	-	6	18	0	0	0	[illegible]	0	0	0	1
1 10 cigarettes daily	0	25	11	0	-	-	6	12	0	0	0	2	0	0	0	1
≥11 cigarettes daily	0	13	0	0	-	-	0	6	0	0	0	0	0	0	0	0
Cigar and/or pipe	0	0	0	0	-	-	0	0	0	0	0	2	0	0	0	1
Total number	8	8	9	9	0	0	17	17	30	30	48	48	2	2	80	80

D=Deceased twin; S=Surviving co-twin

Tabl 28 Smoking habit in mal and femal d ath di ordant pair ac ording to arli r qu ti nnair with pr ent and former moker ombined Perc ntage di tribution

	IHD								not IHD							
	MZ		DZ		XZ		T t		MZ		DZ		XZ		Tot	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
MALES:																
N n smokers	10	20	20	16	20	0	18	15	20	16	26	19	0	0	24	18
T tal mok r	90	80	80	84	80	100	83	85	80	84	74	81	100	100	76	72
Total cigar tt smok rs	60	80	40	36	80	100	50	55	36	52	41	50	100	100	40	52
1 10 igar tt daily	30	30	32	20	60	40	35	25	24	44	-4	36	100	0	25	38
≥11 cigarettes daily	30	50	8	16	20	60	15	30	12	8	17	14	0	100	15	13
Cigar and/ r pip	30	0	40	48	0	0	33	30	44	32	33	31	0	0	37	31
Total number	10	10	25	25	5	5	40	40	25	25	42	42	1	1	68	68
FEMALES:																
N n smoker	75	50	78	67	-		77	59	70	77	85	79	50	100	79	79
Total smokers	25	50	22	33			24	41	30	23	15	21	50	0	21	21
Total igarett smoker	25	50	22	33			24	41	30	23	15	19	50	0	20	20
1 10 cigarett s daily	13	38	22	11			18	35	30	-3	15	19	50	0	15	20
≥11 igarett daily	13	13	0	22		-	6	18	7	0	4	6	0	0	3	4
Cigar and/ r pipe	0	0	0	0			0	0	3	0	0	2	0	0	1	1
Total number	8	8	9	9	0	0	17	17	30	30	48	48	2	-	80	80

D=De ased twin; S=Surviving twin

Table 29 Di tribution of death discordant mal twin pairs according to concordance/discordance with re peot to moking habits (present and former mok r combined)

		Deceased twin															
		IHD								not IHD							
		MZ		DZ		XZ		T		MZ		DZ		XZ		Tot	
		NS	SM	NS	SM	NS	SM	N	M	NS	SM	NS	SM	NS	SM	NS	SM
Surviving co twin	NS	1	1			0	0	3		1	0	4	4	0	0	8	4
	SM	0	8	3	18	1			11	1	0	[illegible]	7	0	1	8	48

Tabl 30 Di tribution of death dis ordant male twin pair a cordi g to on ordance/discordance with re p ct to cigarette habits (pre ent and former smoker combined)

		Decea ed twin															
		IHD								not IHD							
		MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
		NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM
Surviving co-twin	NS	1	1	2	1	0	0	3	2	4	0	4	3	0	0	8	3
	SM	0	8	1	1[illegible]	1	4	2	24	1	14	5	0	0	1	6	35

Table 31 Distribution of death discordant femal twin pairs according to concordance/discordance with resp ct to smoking habits (pre nt and former smokers combined)

		Deceased twin															
		IHD								not IHD							
		MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
		NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM	NS	SM
Surviving o twin	NS	4	0	6	0	0	0	10	0	19	4	35	3	1	1	55	8
	SM	2	2	1	2	0	0	3	4	2	5	6	4	0	0	8	9

SM=Smok r; NS=Non Smok r

MZ twins who died from IHD were cigarette smok rs a again t 80 % of their surviving o twins Counting only pre ent smokers (Table 26) the c rr ponding figures are 30 % v 80 % Smoking >10 igar tt daily i ignificantly ($p<0.05$) more common am ng the surviving male MZ twins who partner died from IHD than among th ir counterpart wh e partners died from oth r cau e ; thi i tru for pre ent smokers (50 % v 4 %) and for pre ent and form r smokers combin d (50 % vs 8 %) The orre ponding omparison betw en the d c a ed mal MZ o-twins giv the ame trend but it i not so pronounc d How ver with regard to th larger group of d ath dis ordant mal DZ pairs there i a higher proportion of cigarette mok r among the surviving o-twins wh e partner died from oth r cau e (50 %) compared to tho e whos partn rs di d from IHD (36 %) but among th ir decea ed partn r th proportion are about the ame (41 % v 40 %)

Of th female twin who died from IHD (Table 28) 14 % were smoker ompared with 41 % of th ir surviving co-twin while th orre ponding figure for th not IHD death dis ordant twins are 21 % and 21 % N arly all th female smoker w re cigarett smok r Of the former smoker only on wa found among th d a ed co twins Non of th differen r orded among the female i tati tically ignificant

Smoking di cordan e among th male death di o rdant pair i sh wn in Tabl 29 30 Only among th DZ twin pair in whom death di ordanc wa not caused by IHD i there an apparent moking di ordan e: in 7 f the smoking di ordant pair the surviving co twins w re smok rs ompared t 4 pairs in whi h th d c a ed twins w r smoker If nly igar tte smoking di oordant pair are onsid red th corre ponding figur f r th not IHD d ath di o rdant pair w re 5 and 3 N r wer ther tati tically ignificant diff renc among th female with r spe t to smoking di ordan (Tabl 31)

Comments

With r gard to mortality from IHD especially in men mo t tudi indicat a po itiv relati n to smoking (Doll & Hill 1964; He t 1966; Hammond 1966; Kann l 1966; Fl t her & H rn 1970) Th pr nt number f smoking di cordant twin pair among th IHD death di ordant pair i too small to p rmit an evaluati n of th ff t f moking on IHD mortality but it can b mentioned that in the mortality tudy on th wh l of th Sw dish Twin R gi try (Frib rg et al 1973) a hyp r mortality partly du t IHD wa found among DZ smoking di rdant pair but n t among MZ pair in both men and women It may well be that igarette moking only r fl t a c rtain onstitution and that

mokers and non-smokers are elf-selected groups This view is also supported by the twin studies by Lundman (1966) and Lilj fors (1970) Examining 196 twin pairs with different intra pair smoking habits Lundman found no difference in IHD manifestations between the more and less exposed twins In the male twin pairs studied by Liljefors smoking habit did not differ appreciably in pairs discordant with regard to the probable presence of IHD Neither did life time exposure, expre ed as cigarette years how any association with IHD Nor was there any difference in smoking exposure in the infarction discordant pairs

The finding of proportionally more cigarette smokers among the surviving male MZ co-twins who e partners died from IHD compared to those whose partners died from other cause could indicate an a sociation between cigar tte moking and IHD The larger male DZ group did not show similar diff rences in cigarette smoking habits However the deceased male MZ twin also displayed the same trend in cigarette smoking habit a the surviving co-twins which may indicate that it is con titutional factors which lie behind these findings

ALCOHOL

tudies n the a sociation between alcohol consumption and IHD have yield d confli ting results Chronic alcoholics have been onsidered to po e s prote tion from coronary ath rosclerosi Several authors (e g Grant et al 1959; Stare 1961; Biörck 1963) found no ass ciation b tween alc h l consumption and IHD Data from th Framingham tudy upport th opinion that alcohol i not correlated with IHD (Kann l 1966) alcoh l consumption apparently showed no ass ciation w th he d vel pm nt of IHD neither did it prote t against atherocl r s of th heart

In a mortality tudy on approximately one million p r ns und r the age of 50 alcoholi m and heavy drinking were overrepr sented am ng fatal IHD cas s (Bainton et al 1963) A study from Norway has also hown a high r mortality from IHD among chronic alcoholi s compar d to the gen ral population (Sundby 1967) In a r port on men born in 1913 registration at the local temperance board showed a strong a o iat on with m rtality in myocardial infarction (Tibblin 1972)

Material e Chapter I

T bl J   Di tributi n of d ath di ordant male twin pair a ording to r gi trati n in Al ohol R gi try

	IHD								n t IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Regi t red	1	0	5	3	1	-	7	5	6	8	19	15	0	0	25	23
T t l numb r	10	10	5	25	5	5	40	40	25	-5	4?	42	1	1	68	68

D=Dec   d twin; S=Surviving co twin

Table 33 Di tribution f d ath di cordant mal twin pairs according to c ncordance/discordance with re pect t registrati n in Alcohol Regi try

		Deceased twin															
		IHD								n t IHD							
		MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
		NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR	R
Surviving co twin	NR	9	1	18	4	2	1	9	6	15	-	16	11	1	0	32	13
	R	0	0	2	1		0	4	1	4	4	7	8	0	0	11	1?

R R gist red; NR N t R gistered

Methods

Persons with alcohol problems were defined operationally as those entered in a nationwide registry for misconduct while und r the in fluence of alcoholic beverage This regi try has exi ted since 1932 All the male death discordant twins w re match d with the r gistry

Results

The numbers of death discordant male twins with entries in the Alcohol Regi try are shown in Table 32 Entries were found for 32 out of 108 (30 %) deceased twins compared to 28 out of 108 (26 %) surviving co-twins Of the 40 twins who had died from IHD 7 (18 %) had b en registered compar d with 5 of th ir surviving partners (13 %) whereas of the 68 who had died from other causes than IHD 25 (37 %) had been r gistered compared with 23 of their urviving partners (34 %) A distribution of the regi ter d twins in the death discordant pairs (Table 33) hows that only one of the IHD death discordant pairs a DZ pair is concordant with respect to registration compared t 12 in the not-IHD group Among the di cordant (registered/not regi tered) pairs there are no ignificant differences within the d ath disc rdant pairs

Comments

An entry in the Alcohol Regi try usually indicates that the person in que tion has alcohol problems It wa found by H lander (1972) that 11 5 % of male twin b rn 1900-1924 had at least one conviction for drunkenness compared to only 0 ? % of the female twins the correspond ing figure for male twins born 1925-1954 being 11 3 % and for females 0 6 % Be au e o few female twins have been regist red only male twins were matched with the Alcohol Regi try A mortality follow up of 9 000 twin pairs from the Sw di h Twin Regi try ha shown entry in the Alcohol R gi try to be a ociated with a higher mortality regardle s of smoking and it wa also found that 10 % of n n-smoker were r gister ed a against 30 % of tho e who smoked more than 10 cigar tte a day (Friberg et al 1973) Concerning the au specific death rat a eak a ociation was f und betw en r gi tration and IHD In the pre ent investigation too r gistration was s mewhat more common am ng the de ceased twin irr p ctive of th cause of death (IHD/not IHD) However alcohol registrat on do not eem to have contributed to the high prevalence of IHD manife tation in the surviving co-twins in th IHD death discordant pairs U ng que tionnaire replie in 1967 con rning

alcohol consumption Myrhed (1974) investigated 70 male twin pairs discordant with respect to alcohol con umption The alcohol discordant pairs did not differ in IHD manifestations evaluated as ST depr ion in connection with exercise A check of the questionnaire replie from 1967/1970 on alc hol consumption for the present material yielded a fairly high proportion of non respondents (27 % among th deceased mal twins and 36 % am ng their surviving co-twins compared to 44 % among th deceased female twins and 46 % among their surviving co-twins) which is why this source of information on alcohol onsumption could not be used h re

PHYSICAL INACTIVITY AND SOME SOCIAL FACTORS

P h y s i c a l i n a c t i v i t y or sedentary living has also been implicated as a ri k factor for IHD (Simborg 1970; Karv nen 1972; Stamler 1973) The finding ar not a learcut or c n i t nt as for many other ri k fa tors probably due to the diffi ulty of defining R sult from th Health Insurance Plan (Frank et al 1966) and the Framingham tudy (Kannel 1966) have shown a triking in crease of fatal myocardial infar tion among physically inactive men N such corr lation wa f und to angina p ct ris and coronary insufficiency in the Framingham tudy but the Health In uranc Plan showed angina p tori to be even mor ommon in th mor active group f men The W t rn ollaborative study (Frank et al 1966) ha al o confirmed that regular ex rcis habit were le common among tho with a fatal a oppo ed to a n n fatal myo ardial infar tion On the ther hand the sev n countrie tudy (Key et al 1970) di clo ed no a sociati n between the incid n f IHD and phy i al inactivity in any of the coh rts It is al o worth noting that the member of the oh rts from Finland had the high st incid n e of IHD but wer usually the mo t phy ically a tive and non obe e

Variou o i a l a n d p y c h o o i a l fa tors hav be n onsid r d a ri ks for IHD (f Jenkins 1971) The complexity of th e variable and th la k of uniform meth d for th ir investigation make such tudi s diffi ult to p rform and evaluat Staml r (1973) c nsid r th mod rn way of life in highly urbanized soci tie to play an important rol in th au ati n of IHD

Results diff r with r gard to th c rrelation of differ nt oci economi group t IHD (Hofv ndahl & Helmers 1973) A high r preva lenc of myocardial infarction ha been noted among employ r

especially owners of small shops and sale men (Biörck et al 1958) Analyses of later studies have tended instead towards a higher prevalence and incidence of IHD in the lower socio-economic groups (Antonowsky 1968)

A low educational background ha also been as ociated with an increased risk of myocardial infarction (Antonowsky 1968; Hinkle 1969) Social group mobility (Wardwell 1968) as well as occupational mobility (Syme 1964) are other sociologic factors which have been found to discriminate IHD patients from other subject

According to Friedman & Rosenman (1959) individuals exhibiting an emotional complex characterized by an excessive sense of tim urgency drive and comp titiveness (type A behavior) are more prone to the onset of IHD than are individuals of the converse type of behavior (type B) Type A subjects usually have elevated cholest rol values and are also more often heavy smokers than type B subject Oth r psychosocial characteristics found in men with myocardial infarction se m to be job dissatisfaction (Sales & House 1971; Theorell 1971) and overtime work (Kasanen et al 1963; Liljefors 1970)

<u>Material</u>: see Chapter I

<u>Method</u>

Information on physical inactivity and som social factors (change of place of work extra work and place of residence) was obtained from questionnaires mailed to all twins in 1967 and 1970 while in formation on education was obtained from questionnaires in 1962 Th twins were asked among other things if they had changed their place of work after the age of 25 and how often if th y had had extra work beside their ordinary job and if thi happened ≥ periodi cally Th y were al o a ked about their place of residenc after the age of 25 and if thi had been mo tly in big cities Higher education is taken here to be edu ation above the compul ory chool including folk-high school vocat onal school and evening courses The twins were also a k d about how much exercise th y had taken when 25-50 years of age; the answer "hardly any was recorded here

<u>Results</u>

The di tribution of death discordant pairs according to their answers on physical ina tivity and various sociologic variabl is given in Tables 34 and 35 Hardly any e rcise when 25 50 year of age is

Ta e 34 Phy inac v y and ome e of variab s n u ma e dea h [illegible]
a rd ng to ari r qu nnair Ab olu e number and preva en ra e in % (par nthe [illegible]

	IHD								no IHD							
	MZ		DZ		XX		Tot		MZ		DZ		XX		To	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Hardly any ex rci	2 (20)	1 (10)	6 (24)	6 (4)	0 (0)	0 (0)	8 (20)	7 (18)	3 (12)	2 (8)	4 (10)	5 (12)	0 (0)	0 (0)	7 (10)	7 (10)
Change of pla of wo k ≥5 times	0 (0)	0 (0)	0 (0)	2 (8)	3 (60)	1 (20)	3 (8)	3 (18)	4 (16)	4 (16)	6 (14)	3 (7)	0 (0)	0 (0)	10 (15)	7 (10)
Extra work	3 (30)	3 (30)	14 (56)	13 (52)	1 (20)	0 (0)	18 (45)	16 (40)	12 (48)	15 (60)	18 (43)	24 (57)	0 (0)	0 (0)	30 (44)	39 (57)
Place of resid nc M tly big cities	0 (0)	1 (10)	4 (16)	3 (12)	1 (20)	1 (20)	5 (13)	5 (13)	4 (16)	2 (8)	7 (17)	10 (24)	1 (100)	1 (100)	12 (18)	13 (19)
Higher education	4 (40)	3 (30)	3 (12)	2 (8)	3 (60)	3 (60)	10 (25)	8 (20)	2 (8)	6 (24)	12 (.9)	9 (.1)	1 (100)	0 (0)	15 (2)	16 (24)
Total number	10	10	25	25	5	5	40	40	25	25	42	42	1	1	68	68

D=Deceased twin; S=Surviving co twin

Table 35 Phy ical inactivity and s me ocioi gic variables in the female death discordant pairs according to earlier questionnaires Ab olute numb r and prevalence rates in % (par nthesis)

	IHD								not IHD							
	MZ		DZ		XZ		Tot		MZ		DZ		XZ		Tot	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Hardly any xer ise	2 (25)	0	1 (11)	0			3 (18)	0	(7)	1 (3)	5 (10)	7 (15)	0	0	7 (9)	8 (10)
Change of pla e of work ≥5 time	1 (13)	0	0	0		-	1 (6)	0	(7)	([illegible])	1 (2)	(4)	0	0	3 (4)	4 (5)
Extra work	[illegible] (25)	0	1 (11)	3 (33)	-		3 (18)	3 (18)	5 (17)	8 (27)	15 (31)	16 (33)	0	0	[illegible] (25)	[illegible] (30)
Place of r siden e Mostly big cities	0	0	0	0		-	0	0	7 (23)	4 (13)	9 (19)	10 (21)	0	0	15 (19)	[illegible] (19)
Higher education	2 (25)	2 ([illegible]5)	2 (22)	1 (11)		-	4 (24)	3 (18)	7 (23)	7 (23)	10 ([illegible]1)	10 (21)	0	1 (50)	17 ([illegible]1)	18 (23)
Total number	8	8	9	9			17	17	30	30	48	48	2	2	80	80

D=Deceased twin; S=Surviving co-twin

reported slightly more often among the IHD death di cordant male pairs than among the not IHD d ath discordant pairs (0 % a 18 % compared to 10 % vs 10 %) Among th IHD death di cordant femal twin pair 3 (18 %) of the d eased twins had reported hardly any exer i vs none of the surviving co-twins With regard to change of pla e f w rk xtra work and plac of residence there ar no sub tantial d ff rence b tween the surviving and the de ased twin in the IHD d ath discordant mal and female pairs None of the f male twins in th IHD death di c rdant pairs had mostly lived in big c ti h r a thi applied t 19 % in the not IHD group With r gard to higher edu at on (above ompuls ry school) th proportion of educat d subjects s much th same in th IHD and n t-IHD male death di ordant pairs but there ar more mark d differ nce within th variou zygo ity groups Among the female IHD death di cordant pair the prop rt n of twin with higher educati n i 24 % v 18 % compared to 1 % vs 23 % in th not IHD group None of the differences i stati tically significant

Comment

The ociologic data in the pr nt study onc rn factor which may possibly predi pos for IHD How ver pre ipitating fa t r such as various life chang have b en shown to pre ed th onset of myo cardial infarction (Th orell 1971) and sudden death (Rah & Lind 1971) but the accumulation of life change prior to the ons t of disease do n t s em to b a sp cific ign for IHD (Rah 1969)

T judg from the result of thi tudy it do s not s m very lik ly that th o ial fa tors re orded o uld b re ponsible for th increa ed preval nce of IHD manif tations in th surviving co twin in the IHD d ath discordant pair In the twin tudy by Lilj fors (1970) ambition and overtime work were noted ignif antly more oft n among th affected partner n th infar t on di ordant MZ pair

It should b born in mind h w v r that in retro pective tudi the disea d subj t often tend t be m re awar of igns and ymptoms asso iated with th di a in qu t on

Table 38 Respondent co twins tabulat d according to the cumulative di tribution of certain defined biometric risk factors

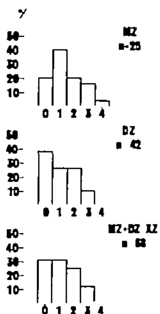
Number of risk factors	MALES IHD MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)	MALES not IHD MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)	FEMALES IHD MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)	FEMALES not IHD MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)
≥1	8 (80)	21 (84)	3 (60)	32 (80)	20 (80)	26 (62)	1 (100)	47 (69)	8 (100)	8 (89)	-	16 (94)	26 (87)	40 (83)	2 (100)	68 (85)
≥2	5 (50)	10 (40)	1 (20)	16 (40)	10 (40)	15 (36)	1 (100)	26 (38)	7 (88)	7 (78)	-	14 (82)	18 (60)	25 (52)	2 (100)	45 (56)
≥3	2 (20)	2 (8)	0	4 (10)	5 (20)	4 (10)	0	9 (13)	4 (50)	3 (33)	-	7 (41)	9 (30)	11 (23)	2 (100)	22 (28)
≥4	1 (10)	0	0	1 (3)	1 (4)	0	0	1 (1)	0	0	-	0	0	1 (2)	0	1 (1)
≥5	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	0
Numb r of co twins	10	25	5	40	25	42	1	68	8	9	-	17	30	48	2	80

T ble 39 R pond nt o twin abula ed a o rd ng o e cumula v a r be i ef r a n efin
biometri and nvir nmental risk factors

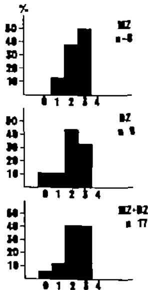
Numb r f risk fa tors	MALES IHD MZ N (%)	DZ No (%)	XZ No (%)	Tot N (%)	MALES not IHD MZ N (%)	DZ No (%)	XZ No (%)	Tot N (%)	FEMALES IHD MZ No (%)	DZ No (%)	XZ No (%)	Tot No (%)	FEMALES not IHD MZ No (%)	DZ No (%)	XZ No (%)	To No (%)
≥ 1	10 (100)	25 (100)	4 (80)	39 (98)	25 (100)	39 (93)	1 (100)	65 (96)	8 (100)	9 (100)	-	17 (100)	26 (87)	46 (96)	2 (100)	74 (93)
≥ 2	8 (80)	18 (7)	3 (60)	29 (73)	22 (88)	28 (67)	1 (100)	51 (75)	7 (88)	8 (89)		15 (88)	23 (77)	32 (67)	2 (100)	57 (71)
≥ 3	4 (40)	11 (44)	0	15 (38)	10 (40)	14 (33)	1 (100)	5 (37)	4 (50)	5 (56)	-	9 (53)	15 (50)	20 (42)	2 (100)	37 (46)
≥ 4	3 (30)	3 (12)	0	6 (15)	4 (16)	5 (12)	0	9 (13)	1 (13)	2 (22)		3 (18)	4 (13)	8 (17)	0	12 (15)
≥ 5	2 (20)	1 (4)	0	3 (8)	1 (4)	1 (2)	0	2 (3)	0	0	-	0	1 (3)	1 (2)	0	2 (3)
≥ 6	1 (10)	0	0	1 (3)	0	0	0	0	0	0	-	0	0	0	0	0
≥ 7	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0
Numb r of o-twins	10	25	5	40	25	42	1	68	8	9		17	30	48	2	80

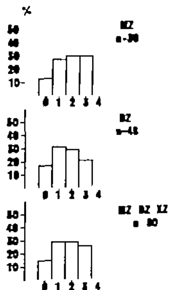
Males





Females

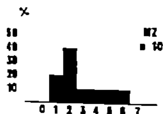


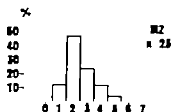


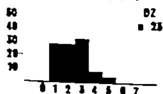
NUMBER OF RISK FACTORS

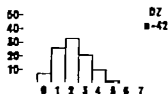
Fig 8 Di tributi n of r spond nt co twins ac rding to certain d biometri risk fa tor Black olumn indicat that th partn r from IHD and white olumns that the partn r died from oth r aus

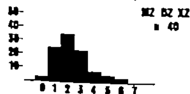
Males

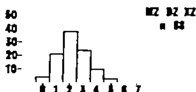




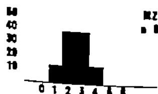


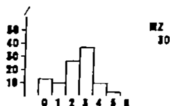


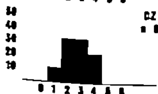




Females













NUMBER OF RISK FACTORS

Fig 9 Di tribution of r spondent o-twins a ording to c rtain defined biometric and environmental ri k factor Bla k olumn indicate that th partn r di d from IHD and whit olumn that th partner died from th r au

None of the differences with regard to the number of ri k factors is statistically significant

Comments

Before combining risk factors it is ne essary to define ri k levels for each factor The cut-off points used here have been chosen fairly liberally which naturally increas the sensitivity but decrease the specificity with regard to the risk of IHD It seem however that the risk rises teadily from the lowest to the highest values for most of th continuous risk factors (Dawber & Kannel 1972; Stamler & Epstein 1972)

New statistical methods for multivariate analy es have been applied more recently to evaluate th ri k of IHD when several risk characteristics are considered simultaneously In the Framingham study a multiple logistic model wa u ed to compute risk probabilities for each individual (Truett et al 1967) Coefficients for each of the risk factors were ba ed on the ri k factor level at the first examination and th 12 year IHD incidence

The relative importance of ri k fa tors is however not the same in all populations Furthermore the multivariate risk functions hav not yet b en translated into clinical terms A combination of risk factors d fined by arbitrarily chosen l vels an therefore be useful as a broad classifi ation of high ri k individuals

In the pres nt tudy th number of risk factor does not differ gnificantly between co-twins wh se partners died from IHD and tho e e partners di d from other causes When only biometric risk fa t rs re combined the ame tendencie are found for both males and females with m re ri k factors among the MZ than the DZ co-twin irrespective of the cause of death of the partner (IHD/notIHD) Furthermore the co-twins with partner who died from IHD have somewhat more risk fa tors than th se whose partners di d from other causes the differen e being more pronoun ed among the femal s

Twin studies cited in connection with the s parate risk fa tors have hown that the biom tric risk factors ar governed to varying degrees by heredity but may also be influ n ed by the environment In a pro pe tive study of 50-y ar old m n in Gothenburg the occurrence of risk fa tor had a predictiv value n t only for the incidence f myocardial infar tion but al o for early d ath fr m other caus (Tibblin 1972) Th differen e in number of risk factor b twe n MZ and DZ co twin irre p tive of th cause of d ath of the partn r might thus reflect constitutional diff ren es Th male co twins with partner

who di d from IHD have rather m re biom tric risk factors than thos whose partn rs died from other cau es but th diff renc i not very marked p sibly because the so called risk factors ar n t as pecific for IHD a used to be thought According to Tibblin (1972) s me common f ctor such as pr mature aging could be re ponsible for an increased number of risk factors a well as the d velopm nt of myo ardial in farction and d ath from non-cardiovascular cau e

Th difference in th number of biometri risk factors show the same trends when nvironmental ri k factor (cigarett smoking physical inactivity and extra work) ar included in the p ibl combination Th environmental fa tor however contribut d propor ti nally l ss to the increased number of factors among femal e p cially the MZ co twin who e partner di d from IHD than among the mal s Harvald & Hauge (1968) in th ir tudy on Danish twin pairs found th o curreno of fatal coronary o clu ion to be g netically d termined to a much larger extent in female than males They consid red environm ntal factors to play a greater part in th eti logy of IHD in males compared to femal

In a compari on between mal and f mal populati ns in Gothenburg with respe t to risk factors for IHD (Bengts on et al 1973) it was ncluded that s x diff renc in th s factor apparently explain nly part of the diff rent in id n e f my ardial infarction in men and women Smoking al ohol nsumption and tr s xp ri n wer however more common am ng th mal

Th pre ent differen in th numb r of risk fact rs do not igni ficantly di riminate the surviving c twins with partn r who died from IHD and thos wh partner di d from th r reas ns which partly could b du t a lack of pe ificity in the risk fa tor Th t nden ci s how v r are nsi t nt with a omewhat high r numb r of ri k fa t rs among the o twin wh e partner died from IHD Th light differen s in th number of ri k fa tor may refl t diff r nt onsti tutions but it is al probabl that gen ti fa tor operat ind p n d ntly f the established risk fa tor in the d velopment of IHD Firmer conclu ion in thi r pe t will have to await a mortality f llow up of th examined twins

IV

GENERAL DISCUSSION

Epidemiological studie have indicated that IHD ha a multifactorial etiology From the standpoint of preventive action most interest has been focu ed on the environmental factors which can be modified and controlled and there have been relatively few studies on the import ance of familial factors and th genetic predisposition for IHD which seem to be especially important for det cting individuals at highe t risk Twin studie probably offer the best tool with which to tudy the relative influence of genetic and environmental factors

Various theoretical models in twin studies were discu sed at an international twin symposium in Puerto Rico in 1969 but it was als pointed out that there i no generally accepted methodology in the de ign of twin studies and that the possibilities are many and complex It wa sugge ted that clinical examination of the partners of twins who have died could be consider d as a valuable extension of mortality studie

One of the main purpo e of the present study is to evaluate a genetic influence in IHD - by examining whether the occurr nce of IHD in the surviving co-twin is as ociated with the partner s cau of death (IHD/not IHD)

Th present material of death di cordant pairs compri es 78 2 % f a sample el cted from the Swedish Twin R gi try This response te mu t be con idered fairly high partly b cause the population drawn from the whole of Sweden and partly in view of the fa t that the co-twins had lost their partner on average 5 months earlier

The accuracy of au e of death determinations depends mainly on a high autop y rate B cause the autopsy rate for the deceased twins in the present material was about 57 % other source of information (hospital re ords etc) were consulted in order to make the mortality evaluation as reliable a possible

This wa e pe ially important becaus th IHD material included cases of sudden death (ISC numb r 795 99) If there was a trong suspicion that a ca e of sudden death had ome oth r explanation than IHD as the und rlying au e then it was not included in the IHD group About 70 % of the udden death included as IHD ca e had eith r a preexisting history of IHD (myo ardial infar tion and/or angina pect ri on effort) or findings at autopsy indicating IHD (igns of fresh or ld infarct and/or evere athero lerotic change of the oronary art ri) as ompared to about 4 % of th twin who died from oth r cau than

IHD. The other 30 % of th IHD sudden deaths had no such earlier evidence f this disorder; they were included because there was no othe reasonable explanation for the death This is in line with ther tudies in which the initial clini al manifestation of IHD was found t be sudden death in about 20 25 % of IHD cases (Kuller t al 1966; Kuller 1966) In th pres nt inve tigation analy e of the occurrence of IHD and risk factors among the urviving co-twins hav been related to the cause of d ath of th partn r (IHD not IHD) In addition to the case of sudden death th IHD death discordant pairs thus in lud tho e where th cause of death was myocardial infarcti n Pooling th se two diagno e a has been done in many epidemiological studies eems to be justifiable not only becaus about 70 % of the sudden deaths howed vidence of previous IHD but also becau the risk fa tors for the sudden death cases are in general agr ement with tho e for myocardial infarction (V din et al 1973) Neither did th relatively mall number of IHD death discordant pairs permit a further br akdown into subgroup

The occurrence of IHD in the surviving co-twins wa e tablished by d termining the prevalence of angina pectoris myocardial infarction and ECG change sugge tiv of IHD Angina pectoris and myo ardial infarction wa found more commonly am ng the surviving co twins who e partners died from IHD than among tho e whose partners died from other causes Among female thi was tru for angina pectori The trend was clear t with regard to myocardial infarction among male but none of the differen es is tati tically ignificant The relativ ly low prevalence of manife t IHD in onjunction with the small number of MZ and DZ co-twins makes it difficult to a s the ignificanc of heredity According to Epst in (1964) a prevalenc rate of 5 5 % for manifest IHD repre nt only the top of the ic berg It has been consid red that ST depre ions in onn tion with exerci could be a fairly sensitive and specific predictor of coronary obstru tive di a e (H lfant et al 1973) The appearance of ST gmental d pr ions in respons to exerci has been shown to be a powerful predictor of overt IHD (Mattingly 1962; Åstrand & Lundman 1968; Blackburn t al 1970; Doyl & Kinch 1970) Using ST d pr ion in onnection with ex r ise a a ign of IHD among the urviving co-twins th frequency among both the male and the femal co-twins who e partn rs died from IHD was ignificantly higher than among the o twins who partner died from oth r aus In the males th difference wa signifi ant for ST d pre ion ≥1 mm but in the femal nly wh n ST d pre ions of ≥0 5 mm w re included The differen c rtainly refl t c nstitutional differenc Th impr ion of a ub tantial geneti influ n in IHD i tr ngthened

smokers especially ≥11 cigarettes daily proportionally more often than the other male MZ and XZ twins a circumstance which may have contributed to the corresponding differ nce in IHD manifestations However the deceased MZ and XZ twins also displayed the same tendencies in the smoking of cigarettes Thus it is pos ible that th differences with regard to cigarette smoking merely reflect differences in constitution which could play the major part also with regard to the development of IHD

Among the men born in 1913 (Tibblin 1972) it seemed that being registered with the local temperance board was strongly as ociated with myocardial infarction All the male death discordant twins in the present investigation have been match d against a register for misconduct involving alcohol Registration was somewhat more common, although not significantly o among the decea ed twins irr pective of the cause of death (IHD/not IHD)

The questionnaire data used in this study on ociologic background variables represent factors which could predispose for IHD These findings however do not preclude the operation of pre ipitating factors such as various life changes which have been shown to pre cede the onset of myocardial infarction (Theor ll 1971) and udden death (Rahe & Lind 1971)

Th high st ri k of developing IHD has b en demonstrated among individuals with multiple risk factors (Dawber et al 1957; Kannel et al 1961; Kannel et al 1967; Tibblin & Wilhelm n 1971; Böttiger & Carlson 1972; Stamler & Epstein 1972) The risk factor used in the combination were defined with fairly liberal cut off points which is a practical and common way of defining risk level Risk evaluation by mean of new statistical methods for multivariate analys s eems to be a more physiological approach considering that the ri k steadily increases from the lowest to the highest value for most of th continuous variable (Dawber & Kannel 1972; Stamler & Ep tein 1972) These method have not yet been translated into clinical terms and the coefficients for each of the risk factors are based upon p cial populations Using a multivariate risk function (Wilh lmsen et al 1973) based on the variable chole terol ystolic blood pre sur and smoking it has been shown (Elmfeldt 1974) that a fairly high proportion of men developed myocardial infarction not withstanding their cla ification a low risks

Wh n only biometric ri k factors were combined in the pres nt study more ri k factors were noted among the MZ co twins DZ co-twins irre pe tive of the cause of death (IHD/not same trend was found when the environmental risk fact rs (

smoking physical inactivity and extra work) w re includ d in the po ibl combination The environmental factors contribut d proportionally le s to the increase in the number of risk fa tors among the femal espe ially the MZ co-twins who e partner died from IHD than among th mal s Environmental factors have been found to play a l s important role in the d velopment of IHD in wom n (Harvald & Hauge 1968; Bengtsson 1973) The differen found in the number of risk fa tors did not significantly discriminate the surviving co-twins wh e partner died from IHD from those wh partn r died from ther reasons a circumstance which could be due to an insufficient specificity of the ri k factor Incid nce data from the pro spective study on men born in 1913 (Tibblin 1972) have shown that mo t f th ri k factors which have been considered to be rather specific for the subsequent occurren e of myocardial infarction also appear to be almo t as valid for early death from whatev r cause

To judge from the result of the pre ent study it an be concluded that a onsid rable genetic influence ems to operate in the development f IHD Gen tic influence are transmitted through om of the established risk fa tors but it is po ible that unknown risk factors or a general biologic factor as premature ageing (Tibblin 1972) or low life potential (Biörck 1974) c nstitute th mo t important unde lying factor in th d velopment of IHD Dawber & Kannel (1972) have propos d that th continued high morbidity and mortality from IHD ould b a consequence of ageing and gen tic make-up and that the important factor contributing to th dev lopment of thi di ease have not been identifi d sati factorily It ha al o been propo ed by Ep t in (1964) that if one could identify and measur all of th pr disposing traits as underlying biologic di turbance in t rms of met boli or other defe t than it would probably emerge that th y are more common than th prevalen e of the di ase would sugge t and show more clear-cut di tributions among family member It wa furthermo concluded that the prevention of IHD demands that th carriers of th s trait b identified that prophyla ti measur can b instituted at an early ag among g n tically sus ptible individual

V

GENERAL SUMMARY AND CONCLUSIONS

All the twins in the Swedish Twin Registry have been the subject of a continuous mortality follow-up ever since the Registry was established in 1959-1961 The complete twin registry is matched regularly against a total death registry for Sweden at the Central Bureau of Stati tics and this procedure makes it possible to obtain the death certificates Since 1971 the matching ha been done every month This procedure was a prerequisite for the present study

The principal object of this investigation was to evaluate the genetic influence in IHD in the examined co-twins and to create a basi for their further mortality follow-up th reby also paving the way for assessments of the predictive value of the m a ured risk factors and the hereditary influence

All unbroken male and female twin pairs from the Swedish Twin Registry below th age of 70 who in the period January 1st 1971 to March 15th 1973 became death discordant were sel cted for the study 78 2 % or 205 of the surviving co-twins could be examined on average about 5 months after the death of the partner

The cause of death of the partner wa e tablished by a t am of physicians on the ba is of all the ass mbled information (h pital records autopsy protocols etc) The cau e of death wer clas ified according to th International Stati tical Clas ification of Dis ase

The zygosity diagnoses were taken from the record in the Twin Regi try which i based upon questions as to similarity If the twins in a pair had given conflicting answ r they were clas ified as unknown zygosity (XZ)

Among 108 male death discordant pairs th cause of death was IHD in 40 pairs (10 MZ 25 DZ and 5 XZ) and other than IHD in 68 pairs (25 MZ 42 DZ and 1 XZ) Among 97 female death di cordant pairs th cause of death was IHD in 17 pairs (8 MZ 9 DZ) and other than IHD in 80 pair (30 MZ 48 DZ and 2 XZ)

191 of the surviving co twins were examined at the Seraphimer Hospital and the other 14 at their local hospital

A sociologic and medical hi tory wa taken using questionnaires The diagno i of angina pectoris was established by interview ac ording to the questionnaire d igned by the London School of Hygiene and Tropical M dicine Myocardial infar tion was considered established when it had be n v rified at ho pital Blood pre sure determinations anthropometric measurement and X-ray of the heart and lungs wer

performed as well a ECG before during and after an ergometer t st Blood sample were drawn after an overnight a fa t for analys s of chol ster l triglycerides uric acid and blood sugar Erythrocyte edimentati n rate hem globin and hemato rit were al o d termined The urine was t ted for proteinuria and glu osuria

Myocardial infarction had o curred in 15 % of the urviving male o-twins wh e partn rs had died from IHD (3 or 30 % of the MZ co-twins and 3 or 12 % of the DZ co twins) a c mpared to only 3 % of th surviving co-twins whose partn rs had di d from oth r ause than IHD Only one of the female o-twins had had myocardial infarction If angina pectori pathologic Q-wave and s gmental ST d pre ions ≥0 5 mm in connecti n with exercise w re also in luded in the crit ria of IHD both male and female co twins (pooled zygo ity groups) ho partner died from IHD di played the igns signifi antly (p <0 05) more oft n than th s who e partn r di d from oth r au In the mal s but not in th female the differenc wa signifi ant even when ST depres ions ≥1 0 mm wa in lud d in the crit ria of IHD Similarly th mal MZ co twins who e partn r died from IHD di played significantly more IHD manife tations (p< 0 05 with ST depre ions ≥1 0mm and p< 0 01 with ST depre ions ≥0 5 mm) than the MZ co-twins who e partners had died from other au Excluding ST depr ions coded in the presenc of high R wave amplitude (Minn ota cod 3 1) which are thought to reflect a l ft ventri ular hyp rtrophy du to hypertension the differences in the female are no long r significant

Mo t of th biom tric factor measured (relative weight skinfold thickne s blood pre ur lipid uric a id) showed mewhat higher valu f r th c -twins who e partn r died from IHD compar d to tho e who partner died for ther rea ns Th diff ren e in ca ual sy t lic blood pre sure wa ignifi ant (p< 0 05) among th femal Overt diab t mellitus sh wed ab ut th ame prevalen in th two d ath di ordant group (IHD not IHD) Nor w re ignificant diff r n es found with regard to hemato rit hemoglobin or erythro yte edimentation rate which om author have d ignated a risk fa tors f r IHD Information obtained earli r through mailed qu sti nnaires about smoking habit physical inactivity extra work chang of plac of work education and pla e of re id nc did not ignificantly di criminat the d ea ed and th surviving o-twins Th IHD d ath di cordant pairs had a om what higher proportion of smok r (83 % v 85 %) ompared t th not IHD (76 % vs 72 %) To smoke ≥11 cigarett daily wa ignifi antly (p< 0 05) more ommon am ng the urviving mal MZ c twins who partners died from IHD than among th wh e partn r died from oth r caus Th c rr sp nding ompari on betw en

the deceased male MZ co twins gave the same trend but not so pronounced

All male death di cordant twins were matched against a nationwid register for misconduct while under the influence of alcohol Registration was more common among the not-IHD death discordant pairs The deceased partners had been registered somewhat more often irrespective of the cause of death (IHD not IHD)

The biometric risk factors (relative weight ≥110 according to a height weight index basal systolic blood pressure ≥160 mm Hg and/or basal diastolic blood pressure ≥95 mm Hg cholesterol ≥250 mg/100 ml and/or triglycerides ≥150 mg/100 ml and overt diabetes mellitus) were then combined with the environmental ri k factors (cigarette smoking extra work and physical inactivity) It wa found that the surviving co twins whose partn rs died from IHD had on average a somewhat higher number of biometric risk factors than those whose partners died from other cause the difference being more pronounced among the female co twins The inclusion of environmental risk factors contribut d proportionally l ss to the number of risk factors among the females especially th MZ co twins

Th conclusions from the present investigation could be summarized as follows:

1) The pre ent results indicate a sub tantial genetic influence in the development of IHD
2) In males the risk factors measured singly and in combinati n seem to explain only part of the differen e in IHD manife tations b tween th surviving co twins wh se partners died from IHD and those who partners died fr m other causes In females how ver elevated blood pressure may explain a great deal of the difference found in IHD manifestations The g netic influences ar probably transmitted n t only through ome of the risk factors measured but also through fa tors which are till unknown
3) The environmental fa tors recorded by earlier qu stionnair s did not discriminate ignificantly between the deceased and the surviving co twin

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APPENDIX

The "Minnesota code" for ECG classification
Adaptation to CR (CH) lead and modification of the code for ECG recorded during and after exercise

Q and QS patterns

(Do not code in presence of ventricular conduction defects 6,4 or 7,1)

1,1,1 Q/R amplitude ratio 1/3 or more plus Q duration 0.03 sec. or more in any of leads I, II, $CR_{2,3,4,5,6,7}$

1,1,2 Q duration 0.04 sec. or more in any of leads I, II, $CR_{1,2,3,4,5,6,7}$

1,1,3 Q duration 0.04 sec. or more plus R amplitude of 3 mm or more in lead VL.

1,1,4 Q duration 0.05 sec. or more in lead III plus any Q wave of at least 1.0 mm amplitude in VF

1,1,5 Q duration 0.05 sec. or more in lead VF

1,1,6 QS pattern when R wave is present in adjacent lead to the right on the chest in any of leads $CR_{2,3,4,5,6,7}$

1,1,7 QS pattern in all of leads CR_1 through $CR_{4,5,6}$ or 7

1,2,1 Q/R amplitude ratio 1/3 or more plus Q duration at least 0.02 sec. and less than 0.03 sec. in any of leads I, II, $CR_{2,3,4,5,6,7}$

1,2,2 Q duration at least 0.03 sec. and less than 0.04 sec. in any of leads I, II, $CR_{2,3,4,5,6,7}$

1,2,3 QS pattern in lead II.

1,2,4 Q duration of at least 0.04 sec. and less than 0.05 sec. in lead III plus any Q wave of at least 1.0 mm amplitude in VF

1,2,5 Q duration at least 0.04 sec. and less than 0.05 sec. in lead VF

1,2,6 Q amplitude of 5 mm or more in either of leads III, VF

1,2,7 QS pattern in all of leads CR_1 through CR_3.

1,2,8 R amplitude decreasing to 2.5 mm or less, and absence of codes 3,2, 7,2 or 7,3 between any of leads CR_2 and CR_3, CR_3 and CR_4, CR_4 and CR_5, CR_5 and CR_6 or CR_6 and CR_7

1,3,1 Q/R amplitude ratio at least 1/5 and less than 1/3 plus Q duration of at least 0.02 sec. and less than 0.03 sec. in any of leads I, II, $CR_{2,3,4,5,6,7}$

1,3,2 QS pattern in absence of code 3,1, in each of leads CR_1 and CR_2.

1,3,3 Q duration of at least 0.03 sec. and less than 0.04 sec. plus R amplitude of 3 mm or more in lead VL.

1,3,4 Q duration of at least 0.03 sec. and less than 0.04 sec. in lead III plus any Q wave of at least 1.0 mm amplitude in lead VF

1,3,5 Q duration of at least 0.03 sec. and less than 0.04 sec. in lead VF

1,3,6 QS pattern in each of leads III and VF

ST depression

(Do not code in presence of ventricular conduction defects 6,4, 7,1,2,4)

4,1	ST J depression of 1.5 mm or more	CR_{2-7}
	or 1.0 mm or more	I, II, VL, VF
	and ST segment straight and slowly ascending, horizontal or downward sloping	
4,2	ST J depression of 1.0-1.4 mm and ST segment straight and slowly ascending, horizontal or downward sloping	CR_{2-7}
4,3	ST J depression of 0.5-0.9 mm and ST segment straight and slowly ascending horizontal or downward sloping	CR_{2-7} I, II, VL, VF
4,4	No ST J depression as much as 0.5 mm but ST segment downward sloping and reaching 0.5 mm or more below P-R baseline	CR_{2-7} I, II, VL, VF
4,5	No ST J depression as much as 0.5 mm but ST segment horizontal or downward sloping but reaching less than 0.5 mm below P-R baseline	CR_{2-7} I, II, VL, VF
4,6	Isolated ST J depression of 1.5 mm or more	CR_{2-7}
	or 1.0 mm or more,	I, II, VL, VF
	ST segment upward sloping	
4,7	Isolated ST J depression of 0.5-1.4 mm or more	CR_{2-7}
	or 0.5-0.9 mm or more	I, II, VL, VF
	ST segment upward sloping	

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Supplementum 579

Cardiac Catheterization

Development of the Technique, its Contributions to Experimental Medicine, and its Initial Applications in Man

By André Cournand

Distributed by The Almqvist & Wiksell Periodical Company Stockholm, Sweden

CARDIAC CATHETERIZATION

CARDIAC CATHETERIZATION

Development of the Technique its Contributions to Experimental Medicine and its Initial Applications in Man

By

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Edited and expanded version of the Jimenez Diaz Memorial
Lecture presented May 19 1970 in Madrid

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CONTENTS

CARDIAC CATHETERIZATION

In considering the successive phases of development of the technique of cardiac catheterization, which in modern times has played an important role in experimental and human physiology as well as in medical practice three separate periods may be identified.

I The first extends from the middle to the end of the 19th century After a probe—a tube of glass or flexible material—introduced into animal hearts was used to solve two controversial problems in cardiac physiology the technique was extended to improvement of recording measurements and knowledge of cardiac events and mechanisms Thus experimental period yielded a considerable amount of information concerning the technique itself the required instrumentation and the validity of the physiological information derived from it Briefly catheterization of the heart provided a strong im pulse to the development of cardiac physiology in various animal species

II The second period started after a lag of 25 years in 1929 with the courageous and repeated attempts by a young surgeon still in training to demonstrate on himself that a ureteral catheter could be passed through an arm vein downstream to the right atrium. After these early tests of the apparent safety of right atrial catheterization in man several investigators utilized the technique as a means of injecting undiluted a radiopaque substance into the right atrium in order to visualize the pulmonary circulatio and heart chambers In addition a few other clinicians among them Jimenez Diaz in isolated attempts and without follow-up employed the catheter to draw samples of mixed venous blood from the right atrium for analysis of some of its chemical contents In particular respiratory gases. Apart from the interest this use of the catheter aroused in the early development of angiocardiography as a clinical method of diagnosis it had negligible influence on, and significance for progress in knowledge of fundamental or applied cardiac physiology during the remaining few years of this period

III The third period, starting in 1936, witnessed the elaboration extension, and universal acceptance of intravascular and intracardiac catheterization as an essential method of physiological investigation in normal and diseased man of diagnosis in cardiocirculatory (acute chronic, acquired or congenital) and pulmonary diseases and of controlled testing and evaluation of a variety of medical and surgical treatments of these diseases This transformation resulted from several developments. (*a*) Design and fabrication of the catheter were improved (*b*) It was found that a vast array of measuring equipment and tracer substances could be producti ely used in conjunction with catheterization. (*c*) It was repeatedly demonstrated that the catheter could be left *in situ* for long periods of time (*d*) The ability to advance its tip into the right ventricle and pulmonary artery opened up the study of the pulmonary circulation in various physiologic conditions and in cardiopulmonary diseases (*e*) The feasibility of advancing the catheter tip into other cardiac chambers and large vessels either through abnormal communications or through channels other than large peripheral veins was demonstrated During the past 33 years access to human intracardiac ca ities and intravascular lumina by means of the catheter led to far reaching developments in physiologic knowledge and medical practice based upon this knowledge

In this essay I shall analyze in some detail the history of the first two periods. The third period is the subject of an independent examination which I am now in the process of completing since it is deserving of as searching a treatment as the previ ous periods Accordingly I shall consider here in some detail the investigations and the plan on which they were based which marked the preceding and early years of this period (1932–1945) but will re strict myself to a few general comments regarding the proliferating applications of cardiac catheterization which characterize the subsequent portions of this period

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1 THE ADVENT OF CARDIAC CATHETERIZATION—CLAUDE BERNARD

Cardiac catheterization was first performed (and also named) so far as can be ascertained by Claude Bernard the nineteenth century scientist who so particularly stressed the importance of physiology for the understanding of disease. A good medical school cannot be separated from the hospital "[1] he wrote reflecting his conviction that the backbone of scientific medicine is experimental physiology integrated with clinical observation.

The problem which evoked the novel procedure of introducing a measuring device into the beating heart in order to make scientific observations concerned the respective temperatures of blood in the right and left ventricles In the 1840 s this was a matter of considerable controversy Several decades earlier Lavoisier had suggested that animal heat is produced as a result of respiratory gas exchange during transit of blood through the lungs.[2] Asserting that respiration is a form of combustion he declared that in the lungs the combination of oxygen with carbon produced carbon dioxide and its conjunction with hydrogen water both reactions being accompanied by the liberation of the element caloric that is heat Subsequently his followers drew the conclusion that the temperature of blood returning to the left heart is greater than that of blood entering the pulmonary circuit.

For some 50 years invocation of Lavoisier s authority was sufficient to support this view In 1837 an alternative was advanced by Gustav Magnus:[3] Combustion takes place in the tissues. He based this claim on the results of experiments in which he showed that in the venous system the carbon dioxide concentration increased whereas the oxygen content declined as compared with the arterial system Later in 1842 according to Bernard Magnus stated that carbonic acid is not formed in the lungs and that combustion therefore occurs in all tissues of the organism

With the issue thus joined the competing hypotheses were vigorously debated From the pulmonary combustion hypothesis it followed that blood entering the left ventricle is warmer than that flowing into the right-sided chamber The tissue combustion hypothesis entailed the assertion that blood coming from the tissues by way of the venae cavae is warmer The first view was upheld by Gay Lussac and Magendie a professor at the Collège de France who were planning experimental work on the relative concentrations of carbon dioxide in arteries and veins These investigators denied the validity of Magnus findings strictly on the basis of their doubt in the reliability of his work. Observations on the temperature difference between the ventricles were made by others but were of dubious validity since the data were obtained from open-chest or dead animals

To determine which of the opposing views was correct, Bernard saw that a simple experiment might be performed—provided that the experimental conditions approximated the normal physiological state as closely as possible. In these circumstances measurement of the temperature of the blood in each of the ventricular cavities would be sufficient to determine which hypothesis was correct

Bernard s initial observations on this problem were made in 1844 when he was serving as an assistant to Magendie The experimental subject was a horse whose exposed carotid artery had been incised Through the opening Bernard introduced a

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[1] Claude Bernard *Leçons sur la Chaleur Animale* Paris, Librairie J -B Baillière et Fils, 1876 p 3

[2] It was among Lavoisier s principal contributions to show that animal heat is produced through a chemical reaction, and not through vital mechanical, or other processes His interest of course was in the relationships among combustion respiration and liberation of animal heat, and not so much in the physiological exploration of these processes, and he granted the possibility that combustion takes place elsewhere in the organism than in the lungs For a discussion of these matters, see Hoff H E Guillemin, R and Sakiz E Claude Bernard on animal heat. An unpublished manuscript and some original notes *Perspectives in Biology and Medicine* 7 347 368, 1965

[3] Magnus, G., Über die im Blute enthaltenen Gase Sauerstoff Stickstoff und Kohlensäure *Ann Phys Chem.* 1., 583 1837

[4] *Op cit* p 23

[5] *Ibid* p 4

[6] *Ibid* p. 47

long mercury thermometer into the left ventricle and recorded the temperature of the blood Then he passed the thermometer through the jugular ein into the right ventricle In repeated observations the temperature was a few tenths of a degree higher on the right side, indicating that the pulmonary combustion hypothesis was incorrect. The temperature difference Bernard noted was lesser under basal conditions than during digestion or exercise.

It was characteristic of Bernard s physiologic work that he returned repeatedly to a given problem sometimes to confirm or to refute the observa tions of other scientists, at other times to repeat the work using better techniques frequently it was Bernard himself who contributed the improved methods. Over a period of 30 years Bernard re turned to the temperature problem not only in 1849 and 1853–1855 but also in 1857 and 1871–1872.

In 1849 before the members of the Société de Biologie Bernard presented an experiment showing that the blood in the hepatic vein is warmer than that in the central aorta. In the period 1853–1855 after G Liebig had reached conclusions similar to his [16] Bernard returned on a number of occasions to intracardiac temperature measurement in dogs and sheep collaborating with Walferdin a specialist in temperature measurement. In these experiments a metastatic thermometer devised by Walferdin was used this instrument could discriminate temperature differences as slight as 0 01°C The results of these studies were published in several texts. In all instances it appears Bernard's original observations were confirmed

Bernard's book *Leçons sur la Chaleur Animale* published in 1876 recapitulates 22 lectures on this subject delivered during the academic year 1871–1872. In the first lectures he reviewed the entire early history of the debate concerning the temperature of the blood in the ventricles. In the fifth lecture Bernard reported experiments in which he used a very sensitive thermoelectric probe a modification of the instrument originally developed by Becquerel[18] like Walferdin s thermometer it allowed differentiation of tempera tures by as little as 0 01°C But what is chiefly of interest in the fifth lecture is Bernard s admirable description of the catheterization technique as applied in intraventricular temperature measurement in a dog:

> An incision is made (in the neck) to expose the jugular vein. It is by this route that right heart catheterization will proceed For this purpose the tip of the catheter is given slight anterior curvature introduced into the vessel and advanced by continuous yet not excessive pressure. Soon we feel a slight resistance. Previous experiments have shown that this occurs when the tip of the catheter has reached the atrium. The occurrence of this resistance therefore provides an important landmark. One must then rotate the probe medially and toward the left while simultaneously withdrawing it short distance. Then, by pushing again, one enters the right ventricle. The goal has been reached.
>
> The second step is to introduce the second catheter into the left ventricle. After exposing and isolating the internal carotid, we introduce the catheter at the same time pulling the vessel upward in order to eliminate the curve that normally is present at the carotid artery s junction with the aorta.
>
> Introduction of the thermoelectric probe into the left entricle cannot always be accomplished with facility Most of the time the sigmoid [i.e., aortic] valves prevent the passage of the catheter s tip which tends to become lodged in the pigeon nest formed by leaflet and the vessel wall One cannot advance into the ventricle without perforating or rupturing the leaflet. □

Ibid p 42. Bernard recorded the fact that his experimental subject, which had been made available to him by Magendie, then chairman of commission on horse hygiene had glanders. This condition was likely not in an advanced stage, however and therefore probably did not distort the interventricular temperature differences, since the horse was able to perform usual activities at the time Bernard studied him

A useful guide to Bernard scientific work is provided by M.D Grmek *Catalogue des Manuscrits de Claude Bernard* (Paris, Masson et Cie, 1967). According to this text, records of some of the numerous studies of the temperature problem appear in Notebooks 7b (1848 1849), 8c (1854 1855), 8f (1853) and 26a (whose 50 folios contain complete transcripts of all experiment on the temperature problem)

Bernard *op cit* p. 42.

[16] Liebig, G *Über die Temperaturunterschiede des venösen nd arteriellen Blutes*. Giessen, 1853

See Recherches sur l'influence que la section du grand sympathique exerce sur la chaleur animale (*Compt rend. de l'Académie des Sciences* 1852; *Leçons de physiologie expérimentale ppliquée à la médecine* (Paris, 1855 ol. I p 196)· Recherches expérimentales sur la température animale (*Compt rend d l'Académie des Scienc* 1856 vol 43)· *Leçons sur les propriét physiologiques et les altérations pathologiques des liquide de l'organism* (Paris, 1859 vol I p 50 *et séq*). For Walferdin description of his instrument, see *Bulletin de la Societé géologique* vol. II, p 83

[18] Becquerel et Breschet. Mémoires sur la chaleur animale. *Annales de ciences naturelles Zoologie* 2e serie, vol III and IV

[19] Bernard *Chaleur Animale* p. 78 The translations of this and the following two passages are not literal at every point and are my own.

After describing the anatomical and functional *characteristics of the aortic valvular leaflets* Barnard goes on.

> In order to introduce the tip into the left ventricle one must wait for the moment when systole begins, as there is then a small aperture between the free edges of the valve leaflets. This is not easy ... However it is easy to feel whether the tip of the catheter has entered the left ventricular cavity or is blocked at its entrance by the valve. In the latter case one sees the probe moving upward with each cardiac contraction. If one holds the proximal end of the catheter between two fingers one feels a single impact and senses that the tip of the catheter has met an obstacle and is not free. In the former case, to the contrary, one feels that the contraction is transmitted more forcefully but that there are two distinct impacts, the first coinciding with systole and produced, without doubt, by the impact of the contracting ventricular wall on the tip which is struck and pushed aside, and the second corresponding to diastole and probably due to the shock of closure of the aortic valve leaflets.
>
> In our [present] experiment fate has favored us, and we have penetrated into the left ventricle ... you can see the proximal end of both catheters moving simultaneously in phase with the ventricular contractions. It is necessary to secure the catheters by means of ligatures [proximal to the portals of entry into the vascular system], both to prevent hemorrhage and to avoid ejection of the tips from the cardiac cavities ... result of ventricular contractions but the ligatures are not applied until the tips have been withdrawn slightly in order to avoid the impact of the contracting wall, as otherwise the tip would injure the endocardium or eventually perforate the muscle, resulting in intrapericardial hemorrhage.[14]

Bernard went on to consider the position of the experimental subject that best avoids passing the catheter along false routes in the venous system.

> In my early experiments on horses I always proceeded with the animal in the standing position.[15]

Later, as Buzzi recounts,[16] Bernard gave a minutely detailed description of cardiac catheterization as an operative technique. His text, published posthumously in *Leçons de Physiologie Opératoire*,[17] presents the elements of the procedures for both venous and arterial catheterization of the heart, as well as for entry into the great vessels.

The temperature problem had been solved in the 1850's by Bernard and other investigators. Yet Bouillaud, a member of Bernard's section of the Académie des Sciences, attacked his conclusions in 1877 at a meeting of the Académie. Bouillaud's argument was based on the principle of authority: What Lavoisier had conceived could not be in error ... Bernard's response[18] to Bouillaud is admirable for its detachment and clarity, as well as its insistence on controlled observation under conditions approximating the physiological as closely as possible. He was able to define a number of factors that might distort findings at catheterization, and he indicated how these variables might be controlled.

Bernard's utilization of the technique of cardiac catheterization was not confined to the temperature problem. He also recognized the possibility of measuring intracardiac pressures by this means. More than 100 years earlier, in 1733, Stephen Hales had had the idea of introducing a glass tube into the venous and arterial systems of a living mare in order to measure pressures.[19] However, he did not perform catheterization of the heart chambers in living, closed-chest animals.

Bernard's purpose in measuring intracardiac pressures was to study the physiology of the nervous system and its influence upon blood pressure.[21] On November 29, 1847, at the Collège de France he performed his first experiment on this problem. Present as observers were Magendie and Rayer. Through the incised jugular vein of a small dog, Bernard introduced a glass tube into the right ventricle. Upon connecting the external end of the tube to a pressure recording system (cardiodynamometer) which had been developed by Poiseuille, he measured the right ventricular pressures; the systolic pressure proved to be much lower than that of the peripheral arteries. At autopsy of the dog, it was seen that the right ventricle had been perforated by the tube, presumably near the end of the experiment, since the final measurements were obviously out of line with the initial and intervening measurements.

Bernard continued his investigations of pressures in the ventricular cavities intermittently during at least the following seven years. His notebooks[22]

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[14] *Ibid.*, pp. 80-81.
[15] Ibid., p. 82.
[16] Buzzi, A.: Claude Bernard on cardiac catheterization. *Am. J. Cardiol.* 4: 405, 1959.
[17] J. B. Baillière et Fils, Paris, 1879.
[18] See Hoff et al., *op. cit.* (note).
[19] *Ibid.*
[20] Experiment 3 in *Statical Essays Containing Haemastaticks* (New York: Hafner Publishing Co., 1964).
[21] Notebook 7f in Grmek, op. cit. (note 8).
[22] *Ibid.*: Notebook 7f, 8f and 13h; the latter text, written in 1877, contains a number of reports of intracardiac pressure measurement. Document 776 in Folio ... states that catheterization of the dog heart creates ... singular disease.

contain records of experiments on dogs and horses in which intracardiac blood pressures were meas ured under a variety of physiological states and types of stimulation.

In scanning Bernard s accomplishments related to cardiac catheterization, I note several features:

1 He saw that solution of a scientific problem might be enabled by the development of a totally new laboratory technique

2. He in fact developed the technique and used it successfully in the performance of experiments that resolved the problem and over the course of time he brought the method to a high level of sophistication.

3 From the beginning he emphasized the im portance of making physiological observations under conditions in which the subject was in the basal state and as close to normal as possible. Recogniz ing inter-species variability he used animals of var ious species in his efforts to obtain experimental data

4 He brought a variety of experts from other fields into his research when this seemed fruitful significantly enhancing the quality of the observa tions by doing so

5 Finally he was the first to measure right ventricular pressures in the living animal thus opening a new field to scientific investigation

2. INTRAVENTRICULAR PRESSURE RECORDING

A The cardiac apex beat—Chauveau and Marey

It would be refreshing to linger with Bernard and his work for the physiologist might be surprised to find attitudes that be imagined to be quite contemporary set forth with great clarity and logical rigor in the work of this outstanding scientist The requirements of even an overview of the history of cardiac catheterization demand however that we go on to the next important instance of its application Again the incentive was provided by a physiologi cal controversy—i this instance concerning the nature and timing of the cardiac apex beat This problem and its cognate the question of the varia tions of the apex beat in disease have played an important role in cardiac semiology

Two centuries previously Harvey had taken the position that the impulse felt in the apical region of the thorax was due to ventricular contraction and therefore started with ventricular systole[22] Harvey s opinion was later upheld by Laennec[23] Nevertheless controversy arose as to it exact tim ing (*vide infra*) Understandably its resolution wa of interest not only to physiologists but t physi cians as well In 1861 two French investig tors A Chauveau and E. J Marey addressed themselve to an experimental approach to the problem

Chauveau was a professor of veterinary medicine in Lyons and an experimental physiologi t with a particular nterest in the relationship between heart sound and cardiac physiologic events In 1856 Chauveau and his colleague 1

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[22] In *De Motu Cordis* chapter II p. 28 (translated by Chauncey Leake, Tercentenary Edition Charles C Thomas, Publisher Springfield Illinois 1928), Harvey refers to "three significant features to be noted in the motion and in the period of movement of the heart" and describes the first of these features. "The heart is lifted and rises up to the apex so that it strikes the chest *at that moment* [ventricular systole] and the beat may be felt on the outside

Milne Edwards, in a report on two presentations by Chauveau and Marey (*vide infra*) published in *Compt rend des Séances de l'Académie de Sciences*, vol LIV April 28, 1862, p 899 refers to Harvey observations in the following terms.

"The illustrious Harvey studied thi phenomenon with his usual insight in order to discover its mechanism he made number of experiments in animals, and took advantage of very rare pathological case which enabled [him] to observe directly the heart of young man through gaping aperture in the thoracic anterior wall. The facts observed led him to attribute the apical thrust against th sternum and the ribs t the stiffness of the heart muscle wall associated with ventricular systole and to move ment of projection depending also on this ventricular systole.

[23] The key to the source of Milne Edward's statement (note 23) was supplied t me by Dickinson W Richards few weeks before his death. I "De Generatione Animalium and the Final Assessment (chapter 10 of the book by Gweneth Whitteridge *William Harvey and the Circulation*, published by McDonald, London, and American Elsevier Inc., New York in 1971) appears description of Harvey observations of the beating heart of the eldest son of the Viscount Montgomery

[24] In Chapter II of Volume II p 207 of the *Traité d'Auscultation Médiate* (Brossin et Chaude, Paris 1819) R. T H Laënnec stated, "The impact of the heart (usually felt only in the precordial region) corresponds with the beginning of the systole of the ventricles. Laënnec clearly distinguished this phenomenon from the somewhat similar appearance rarely produced by atrial contraction.

published a book describing their investigations of the cardiac movements and normal sounds [26] Working at the School of Veterinary Medicine in Lyons they chose as experimental subject the horse whose slow heart rate facilitates direct observation.

Chapter IV of Chauveau and Faivre s text contains an analysis of the apical thrust, that is its timing and nature In describing their predecessors work, they cited the theories of Beau, Senal Hunter Beclard *Borelli, Berard Ponchappe Hope Fallon*, and Hiffelstein declaring that all were incorrect. In the view of Faivre and Chauveau "The apical thrust corresponds to the instantaneous change of the ventricular form and consistency at the beginning of systole [which is] transmitted to the entire anterior thoracic wall but felt only where there is no bony structure." Thus their conclusions were in agreement with those of Harvey and Laënnec [27]

E J Marey a young and brilliant Parisian physician subscribed enthusiastically to a concept promulgated by Bernard namely that experimental physiology is a key to the understanding of pathological phenomena Prior to his collaboration with Chauveau Marey had already devised a sphygmograph which could record changes in the pressure of flowing blood In his doctoral thesis [28] he described studies of arterial pulse transmission and offered a physiological interpretation of the meaning of the dicrotic notch observed in the pressure waveform recorded in peripheral arteries.

The source of the idea of introducing a pressure recording apparatus into the heart to study the cardiac apex beat is not clear It is possible that *Chauveau and Marey were aware of Bernard s* work and were encouraged by it to attempt pressure measurement in the cardiac cavities Nor can we trace here the origin of the insight that tracings of intracardiac pressures could be related to cardiac physiologic events Suffice it to note that it was generally accepted in French scientific circles in the early 1860 s that systolic contractions caused increased intracavitary pressures and that changes in these pressures could be deduced from changes in appropriately obtained pressure tracings. To demonstrate the timing of the apical beat, it would suffice therefore to display its time course on a scale which also portrayed cyclic variations in ventricular and atrial pressures To this at that time formidable problem in technique Chauveau and Marey directed their efforts

In their first note on the cardiac apex beat (read before the Academy of Sciences) [29] they described a method of pressure recording using a catheter connected to a sphygmograph As Chauveau and Marey acknowledged in a later report the idea of connecting a system of levers to an instrument for continuous graphic recording must be credited to Karl Vierordt of Tubingen this student of Ludwig evidently had constructed a sphygmograph to record the arterial pressure pulse In Chauveau and Marey s arrangement flexible rubber ampoules were attached to both ends of the catheter which was filled with water One end of the catheter was introduced into a heart chamber the ampoule at the external end was connected to the recording lever of the sphygmograph [30] Another system of tubing with ampoules led from the cardiac apex to a similar device for recording of the apical beat. This approach failed "because of the resistance caused by inertia and friction of the long column of fluid within the tube" [31] and no doubt the elasticity of the rubber ampoules

Therefore Chauveau and Marey had recourse to another transmitting device They were familiar with a different technique for recording intraluminal

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[26] *Nouvelle Recherches Experimentales s les Mouvement et les Bruits Normaux du Coeu Envisagés du Point d Vue d la Phy iologie Medicale* J B Baillère et Fils Paris, 1856.

[27] Chauveau and Faivre also deserve credit for the first experimental recording of pulmonary artery pressure as described in thei book. They obtained this measurement with a needle In Chauveau s subsequent work he evidently never succeeded in advancing a catheter beyond the right ventricle

[28] *Recherches sur la Circulation du Sang* Paris, 1859

[29] Détermination graphique du rapports du choc du coeur avec les mouvements des oreillettes et des ventricles Expérience faite à l aide à un appareil enregistreur (sphygmographe) *Compt rend des Séances de l'Academie de Science* LIII p. 622 October 7 1861

[30] See p. 273 in Appareils et Expériences Cardiographiques. Demonstration nouvelle du mécanisme des mouvements du coeur par l'emploi des instruments enregistreurs à indications continues *Mémoires d l'Academie de Médecine* 26, 768 1863 The great care exercised by Chauveau and Marey in attributing priority to Vierordt for construction of the sphygmograph is noteworthy elsewhere, with H Zuckerman I have advanced the view that recognition of priorities is one of the fundamental principles of the operating code of the scientist (*Studium Generale* 23 941 1970)

[31] See *Gazette Médicale de Paris* May 18 1861 and Chauveau and Marey Détermination graphique (note 29).

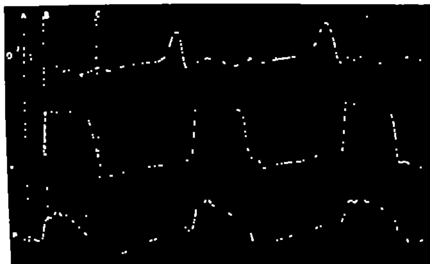


FIG. 7 Simultaneous recording of blood pressure in the left atrium and right ventricle and of the apical beat

Fig 1 Simultaneous recordings (from bottom to top) of apical thrust and right ventricular and right atrial pulse pressures—Chauveau and Marey See text and note 33

pressures developed by Pierre Charles Buisson and described in his M.D thesis.[31] This system consisted of a metal cup closed by an elastic membrane with a side connection to a long tube whose end led to a small metal funnel also closed by an elastic membrane A stopcock allowed introduction of air into the system which could be used to record cyclic events.

Chauveau and Marey modified Buisson's system for their own purposes. Two long tubes bound together one longer than the other by approximately 12 cm and each closed at both extremities by a thin rubber balloon inflated with air were introduced through the jugular vein of an unanesthetized horse.[32] They described at length the tracings they obtained and the timing of those pressure changes that were related to the impact of the apical heart beat They concluded that it is unnecessary to emphasize further the significance of these tracings which seem to demonstrate in an irrecusable manner that the apical beat is an effect of ventricular systole" (Figure 1)

Several months later they published a second note[34] to answer a critique leveled by Beau. The latter tenaciously holding to his theory wondered how it was that the effect of auricular systole during ventricular diastole was not reflected in their tracings, an effect to which he attributed the beginning of the ventricular movement (diasto-systole) preceding its contraction. In their answer they stated that by refining

> the sensitivity of the recording system and by increasing the amplitude and the speed of the tracing we were able to identify during the ventricular diastole all the accessory movement imparted to the blood in the [ventricular cavity] by the auricular systole (characterized by a small undulation of the ventricular tracing or even by the clapping of the valve).[35]

According to a commendable tradition, the Academy of Sciences designated a committee to report on the two notes presented by Chauveau and Marey Flourens Rayer and Claude Bernard were members of the committee with Milne Edwards, who wrote their report. This document[36] was

[31] *Quelques recherches sur la circulation du sang à l'aide d'appareils enregistreurs* Faculté de Médecine de Paris, 1862 For another description of this device see the report by Milne Edward (note 23).

[32] See *Gazette Médicale de Paris*, May 18 1861 also Détermination graphique (note 29).

[33] Détermination graphique des rapports du choc du coeur avec les mouvements des oreillettes et des ventricles (deuxième note). *Compt rend des Séances de l'Academie des Sciences* LIV p. 32, Jan. 6, 1862.

[34] *Ibid*

[35] *Op cit* (note 23).

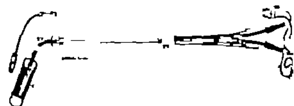


Fig 2 Double-lumen catheter: Chauveau and Marey's modification of Buisson's recording system. See text and notes 32 and 38.

published three months after the presentation of the second note and includes a description of the experiment on a horse which was carried out before the committee by Chauveau and Marey at the School of Veterinary Medicine of Alfort (near Paris). This report, constituting a model of presentation of a physiological problem includes its historical background describes in great detail the instruments used (sphygmograph and tubing) and the technique and discusses the results of the experiment. In their conclusion the committee accepted as undoubtedly proved correct the theory initially advanced by Harvey.

B First systematic description and interpretation of intracardiac pressure recordings (1863)—Chauveau and Marey

In 1863 Chauveau and Marey presented what is perhaps their definitive report on the entire problem of intracardiac pressure recording and its interpretation.[37] The report gives full details of the design construction and operation of the recording system. The report is furthermore excellently illustrated and contains several reproductions of intraventricular pressure recordings. Analysis of their tracings demonstrated (1) an interval of 0 sec from the beginning of atrial systole to the onset of ventricular systole and (2) a 0.1-sec duration of atrial systole as compared with a 0.4 sec duration of ventricular systole. Chauveau and Marey also achieved the first recorded simultaneous measurement of pressures in the left ventricle and in the central aorta. They attempted to define the influence of left ventricular systole upon the contour of the central aortic pressure curve. Moreover they were the first to refer to what later has come to be known as the isometric phase of left ventricular contraction. Their text goes into important detail regarding numerous other facets of cardiac pressure tracings in health and experimentally simulated disease which we cannot consider here. This work unquestionably is a milestone in the physiology of the heart.

In a separate publication[38] a now classical book published in the same year as their joint report, Marey set forth in detail the procedure utilized in these experiments describing the double-lumen catheter (Figure 2) the recording instruments and the tracings (Figure 3) with ample illustrations and legends. In this book he also described a special catheter to be introduced by the carotid artery route with which Chauveau and he secured pressure pulse tracings of the left ventricle (Figure 4). Moreover on the basis of carefully calibrated curves (and giving particular attention to temperature conditions in the heart since blood pressure pulses are transmitted through a gaseous medium) Marey derived average figures for right atrial right ventricular and left ventricular systolic pressures. These were respectively 2.5 ?7.0 and 128.0 mm Hg—results which approximate figures subsequently reported in dog and in man.

Anticipating as it were the concerns of later physicians in a footnote of his book Marey declared

> One can be reassured of the innocuity of this method by examining the horse who is scarcely disturbed walks and



Fig 3 Simultaneous recordings of pulse pressures (from top to bottom) in right atrium, right ventricle, and left ventricle by Chauveau and Marey. See text and note 38.

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[37] "Appareil et Expériences Cardiographiques (note 30)

[38] *Physiologie médicale de la Circulation du Sang*. Delahay Paris, 1863



Fig 4 Special catheter designed by Chauveau and Marey to be introduced into the carotid artery for recording left ventricular pulse pressures (see text and note 39).

eats as usual In only a few instances is the pulse rate slightly increased especially at the time of the introduction of the catheter within the heart cavities.[illegible]

In the Preface to his book Marey emphasized the importance of extending the limits of our senses in physical examination of patients and listed many cavities and canalicular systems which may be explored with catheters and specula in the human. One cannot but wonder Why did not Chauveau and Marey extend their experimental method to the exploration of the circulation in man? Even a tentative answer to that question must take into consideration the overwhelming interest in the role of clinicopathologic studies in the advance of clinical medicine in general and of knowledge of cardiac diseases, in particular This attitude was in stark contrast to the physiological approach to the improvement of diagnostic and therapeutic procedures which was to wait for passage of another three-quarters of a century

In summary Chauveau and Marey were the first to systematically employ catheterization for the investigation of intracardiac pressures The original features of their collaboration in the solution of the controversy regarding the origin and timing of the cardiac apex beat were to establish experimentally the exact timing of atrial and entricular contractions, and their effects on both the movements of the heart and intracardiac and central aortic pressure recordings.

C Critical discussion of the work of Chauveau and Marey on intracardiac blood pressure recording

From 1864 on until the end of the 19th century several physiologists in Germany and England contributed importantly to determination of the validity of the work referred to in the previous section.

1 Adolph Fick (1864) A physicist and physiologist, Adolph Fick was one of the first students and the prosector of Carl Ludwig in Zurich he served in this capacity at the Institute of Physiology from 1852 until 1870, when he was promoted to professor of physiology in Würzburg where he remained until the end of his active life. While in the Zurich Institute laboratory he developed a pressure recording device[illegible] based on a principle different from that of the sphygmograph It involved a 'C' spring whose movements were transmitted through a lever to a feather which graphically inscribed its motions on the kymograph invented by Ludwig.

This recording system was improved further after Fick moved to Würzburg.[illegible] With his model connected to a catheter filled with a bicarbonate solution with a lateral opening near the tip of the elastic tubing Fick undertook a study of the form and level of blood pressure variations in the right and left cardiac cavities and published the results.[illegible]

[illegible] *Ibid*

[illegible] Fick, A. Ein neuer Blutwellenzeichner. *Arch. f Physiologie* 1864 p. 563 For the proximal balloon connected t Marey sphygmograph an lastic, hollow "C"-shaped spring was substituted which uncoiled as air pressure increased within the tubing introduced into the heart.

[illegible] It is noteworthy that Ludwig failed to refer to the ballistic galvanometer invented before the kymograph by Pouillot. Surprisingly also, he did not acknowledge the invention of the U manometer by Poiseuille.

[illegible] Eine Verbesserung des Blutwellenzeichners *Arch. f die gesammelte Physiologie* 30 597 1883 The improvement consisted in the replacement of the "C"-shaped spring by straight steel spring and an air transmitting tube with terminal elastic balloon.

[illegible] Ueber die Schwankungen des Blutdruckes in verschiedenen Abschnitten des Gefass-Systems (Zum Theil nach Versuchen von D Vaceff aus Belgrad in Russlalen). *Verhandl der Wurzburg Physiol. Med Gesellsch.*, N B IV 1873 p 223 See also New York Academy of Medicine Library Bound Pamphlet 570 *Arbeiten aus den physiologischen Laboratorium Wurzburger Hochschule 1872–1878*

Fick's main contention in this report was that this recording system was superior in fidelity to that employed by Chauveau and Marey. He claimed to have discerned a number of artifacts in their published tracings and he criticized their methods for scaling the curves. His arguments, however, are of questionable validity, as becomes manifest on considering several of his conclusions. (*a*) His method records blood pressure "directly." (*b*) The atrial pressure curve is almost horizontal with minimal waving at a level of approximately 0 mm Hg during the whole of diastole: "Is so gut wie constant and naher gleich nul." (*c*) The idea that there can be as much as a 10 mm Hg pressure difference between the minimum and maximum atrial pressures is considered out of the question. (*d*) An elevation of pressure just before the onset of ventricular contraction is unrelated to atrial systole.

Striking as these distortions were, it was in the comparison of left ventricular and aortic peak levels during systole that Fick's technique was most flagrantly at fault. The flat peak systolic tracing of the left ventricle was much lower and of different contour from that of the corresponding and almost simultaneous curve in the aorta. Obviously the lateral opening of the catheter used to record left ventricular pressures was obstructed during systole. To explain this gross discrepancy and paradoxical finding, Fick discoursed at length, a surprising show of dialectic in a scientist who gave so many proofs of objectivity and scientific rigor.

In the same bound pamphlet which contains all the reports of physiologic work from 1872 to 1879 in the Würzburg Hochschule appeared a paper by Badoud, a student of Fick, which refers to the effect of spinal cord section on pulmonary arterial pressure variations as measured using the same recording system.[44] The title was deceptive. In that the work described pressure recording in the right ventricle. Furthermore, the conclusion that the pressure drops in ventricular systole are due solely to transection of the medulla is questionable, since the drop in systolic pressure in the carotid artery was of considerably greater magnitude.

2. Porter, Rolleston, Roy and Adams. In the following years cardiac catheterization gained importance as an investigative technique. New manometric systems were developed, and by the late 1880's the method was routinely used in physiological laboratories for measuring intracardiac pressures, particular attention being given to their relation to cardiac physiological events. There were, however, disputes concerning the possible influence of the catheter on signals transmitted from the cardiac cavity to the manometer.

Porter,[45] in his studies on the filling of the canine right heart cavities, used a single- or a double-lumen rigid catheter made of silver-plated brass, of 2.5 to 3 mm internal diameter, and connected by rubber tubing of the same diameter to the manometric recording system. The total length of the connecting tubing was 30 to 40 cm. With this system he defined the pressure gradients between the right atrium and ventricle. In discussing his technique and the validity of his results, Porter indicated that he was well aware of prior work by Rolleston and by Roy and Adams, who both had rejected the cardiac catheter as a means of faithfully transmitting cardiac events to the manometer.

Rolleston[46] had emphasized the role of friction along the tube and connectors in causing artifacts, but he admitted that the friction through a narrow tube of course is less in the case of a large animal such as a horse (the preferred subject in Chauveau and Marey's experiments) than in the instance of small animals such as dogs and rabbits. He concluded as follows: "In these animals it seems absolutely essential to connect the heart with the recording system by some other method which is free from the objections above stated."[47] C. S. Roy and J. G. Adams[48] were also critical of the technique introduced by Chauveau and Marey and of the validity of endocardial pressure tracings obtained with a catheter. Indeed, they substituted for the catheter a short cannula introduced through the apex of the heart and connected its distal end to a piston manometer filled with oil.

Regarding the argument relating to friction, Porter had this to say:

> The friction between the oil and the wall of the piston manometer is certainly great, when the loss from this

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[44] Badoud, Emil: Über den Einfluss an den Hirns auf den Druck in der Lungen Arterie. New York Academy of Medicine Library, Bound Pamphlet 570: *Arbeiten aus den physiologischen Laboratorium ... Würzburger Hochschule, 1872–1878.*

[45] Porter, W. T.: Researches on the filling of the heart. *J Physiol.* 13: 513, 1892.

[46] Rolleston, H. D.: Observations on the endocardial pressure curve. *J. Physiol.* 8: 235, 1887.

[47] *Ibid.*

[48] Roy, C. S., and Adams, J. G.: Heart beat and pulse wave. *Practitioner* 44: 81 and 44: 161, 1890.

source is added to the loss from friction in the catheter and connecting tube it may well be that the total effect introduces an error in the curve: that the fault is with the catheter and tubes, I can by no means concede.[29]

And he went on to discuss critical damping of which he approved

D Concluding remarks on the validity of intracardiac pressure recording

This controversy among physiologists who were concerned to obtain tracings that correctly and precisely reflected intracardiac and intravascular pressure variations was settled on a truly scientific basis shortly after the end of the 19th century From 1903 on Otto Franck published a series of fundamental and classical papers in which he laid down the mathematical and theoretical basis of faithful reproduction in form, fine detail, magnitude, and timing of intravascular and intracardiac events.[30]

According to Franck s formulation and theoretical principles, recording devices must be constructed so as to have definite characteristics. (*a*) low effective mass of the manometer and mobility of the fluid in the entire connecting and recording system, and (*b*) high coefficient of elasticity of the membranes (rubber or metal alloy). In addition, it was desirable that the optical recording system attached to the membrane provide high magnification. Construction of the system according to the first two requirements entailed that the natural frequency of the entire system extending from the membranes to the mouth of the tubing or cannula in the blood vessel (or cardiac cavity) would exceed, by a factor of at least four the frequency of the fastest component of the inscribed curve (whose frequency can be as great as 25/sec) Franck postulated also that the quality of the recordings with regard to amplitude, phase relations, and fine detail might be improved by critical damping (almost to the point of but avoiding aperiodicity)

The development of pressure recording in man by means of intracardiac or intravascular catheterization witnessed substantial efforts in order to satisfy these fundamental principles

3 MEASUREMENT OF BLOOD FLOW

We now turn to a new chapter in the early history of cardiac catheterization, the discovery of the quantity of blood ejected by the ventricles per unit of time—that is, the cardiac output.[31]

A. Fick s principle

Adolph Fick, shortly after his promotion to professor of physiology at the Hochschule of Würzburg, presented a brief and now famous note before the Society of Physiology and Medicine of this university town in 1870 The note is entitled "Über die Messung des Blutquantums in den Herzventrikeln"[32] (Figure 5) As it stands the title is misleading, since it suggests the measurement of the ventricular blood volume but in the *first* sentence of the report it becomes clear that the measurement refers to the volume of blood that is ejected with each systole

In this note, about one printed page in length,[33] Fick states that *the volume of blood flowing through the lungs per minute can be calculated by dividing the arteriovenous difference of oxygen or carbon dioxide—the amount of gas taken up or given off by a unit volume of flowing blood—into the total volume of the same gas taken up or given off by the lungs per minute* This determination re-

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[29] *Op cit*

[30] Tw of the most important of Otto Franck papers are: Kritik der elastischen Manometer *Z. Biol.* 44 445 1903 and Die Puls in den Arterien. *Z. Biol.* 46:441 1905 Additional references to other work by Franck (also published in *Z Biol.*) and remarkable historical and critical analysis of pressure pulse recordings may be found in Carl J Wigger's text, *The Pressure Pulse in the Cardio-Vascular System* (Longmans, New York, 1928).

[31] A first attempt to estimate the cardiac output must be attributed to Stephen Hales. At the end of the third experiment reported in *Haemastaticks* (*op cit* note 20), whose purpose was measurement of the blood pressure in mare, he bled the animal to death, then filled its left ventricle with melted beeswax. The volume of the solidified wax multiplied by the normal resting heart rate gave figure of 6 liters per minute for the cardiac output. This figure is obviously too small for an animal the size of a mare I attempting to account for this, one notes that Hales ignored the probable reduction in the size of the cardiac cavity after exsanguination, Hales reasoning also presupposed complete emptying of the entricle during systole. H must have felt confident in his measurements, for table in *Haemastaticks* gives estimates of the cardiac output of dogs, oxen, sheep, and men.

[32] *Phys -med Ges Würzburg* July 9 1870

[33] An English translation of Fick note appears in the chapter entitled "Output of the Heart in the book edited by A P Fishman and D W Richards, *Circulation of the Blood—Men and Ideas* (Chapter 2 Oxford University Press, New York, 1964 p. 96).

[illegible]

Fig. 5. The first page of the *Proceedings* describing the Fick principle. See text and note 39.

quires the simultaneous withdrawal of samples of arterial and venous blood for analysis of their gas content and the measurement by a simple gasometric method of respiratory gas exchanges in the lungs. Although probably implied, it is not stated unequivocally in this note that venous blood stands for *mixed* venous blood, that is, for a representative mixture of the venous blood returning to the heart, which can be obtained only from the right heart or the pulmonary artery.

Fick's principle, enunciated with regard to respiratory gas transport and blood flow, is a particular case of the more general principle of dilution upon which are based most methods for measuring blood flow, whether limited to a regional or extended to the total circulation, and whether based on inhaled foreign gas, intravenously infused dye or radioactive tracers.

B. Application of the Fick principle

Fick himself never reported any experiments on animals in which respiratory gas exchange in the lungs and arteriovenous respiratory gas differences were simultaneously determined. But he taught this principle to his students with examples, as can be identified in several editions of his *Compendium der Physiologie des Menschen*.[34] His estimations of cardiac output, it is interesting to note, are reported in the chapter on respiration, and not in a preceding chapter on cardiac work.

Sixteen years elapsed before this principle was applied to the measurement of blood flow in six dogs by Grehant and Quinquaud. In their report to the Societé de Biologie[35] they described the procedure by which they simultaneously secured mixed venous and arterial blood in equal amounts, the former from the right heart by means of a catheter introduced by the venous route and the latter from a cannula placed in the carotid artery. Then they determined the weight of the CO_2 exhaled in 1 minute and the CO_2 content of the blood samples using two vacuum pumps.

The most important application of the Fick principle and of the method of cardiac catheterization was yet to come. The contribution of Nathan Zuntz and his associate O. Hagemann constitutes a landmark in our account; its importance and significance stem from the quality of the scientific methodology and the care taken in details of technique and experiments in this first attempt to combine blood flow measurement with direct recording of

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[34] Braumüller, Wien. In Chapter 7 of the second edition, published in 1874, Fick tabulated arteriovenous differences for nitrogen, oxygen, and carbon dioxide, the average values in dogs being 5.35 vol. % for oxygen and 4.49 vol. % for CO_2. In the fourth edition of this text, published in 1891, higher figures are presented: 7.3 vol. % for O_2 and 5.[illegible] vol. % for CO_2. On page 311, assuming a CO_2 output of [illegible]0 ml/min and an A-V CO_2 difference of 5.8 vol. %—a figure based on the average of very numerous ("zahlreichen") measurements in dogs—Fick arrives at a reasonable although low value for cardiac output in man, namely 4600 cc/min.

[35] Grehant, N., et Quinquaud, C. E.: Recherches expérimentales sur la mesure du volume du sang qui traverse le poumon en un temps donné. *Comp. Rend. de S[é]ances hebdomadaires de la Soci[été] d[e] Biologi[e]*, tome 5, série 8, 1886, p. 159. The calculated cardiac output in 6 dogs whose weight ranged from [illegible] to 18 kg varied between 0.591 and [illegible].614 liters per minute or 84 and 16[illegible] [illegible]/kg body weight per minute.

blood pressure in the pulmonary artery, aorta, or both for the determination of cardiac work.

Zuntz, formerly a student of Pflüger, started in 1886 and terminated in 1894 extensive physiologic studies of respiratory gas exchanges, heat production, ventilatory work, cardiac output and work, and systolic ejection velocity in horses at rest and during exercise. A detailed compendium of these studies forms the basis for a monumental monograph published in 1898.[56] Chapter IX of this monograph supplies detailed information regarding the correct technique for measuring blood flow according to the Fick principle: after introducing a ureteral catheter, well oiled to prevent blood clotting, into the right atrium, Zuntz insisted on the essential procedure for validating results: simultaneous collection of expired air and of both mixed venous and arterial blood in equal amounts over a period of 1 1/2 to 2 minutes with the experimental subject in a stable state. In several tables he compared the gas exchange and blood respiratory quotients. In table LXXX he averaged the blood flow values measured in all his experiments. It amounted to 29.55 liters per minute for an average weight of 347 kg, i.e., 84 cc/kg/min. The scientific quality of his work matched his scientific integrity. He acknowledged that as he started measuring blood flow in horses in 1891 and reported his application of the Fick principle using the term "new method"[57] he was not aware of Grehant and Quinquaud's priority. He also frequently referred to the study of cardiac pressure by Chauveau and Marey and, using the time course of their curve for calculating blood systolic ejection velocity, compared his calculations of blood velocity with those obtained previously in the horse by Chauveau et al.,[58] i.e., 68 cm/sec versus 57 cm/sec.

Zuntz, indeed, may be considered a real precursor in combined studies of respiratory gas transport and hemodynamics, heralding a period 50 years hence when such combined studies were performed on a larger scale in normal and diseased man.

C. Validation of the Fick principle in its original formulation

The validation of the Fick principle also had to wait for another half century, since in his simplified formulation Fick had examined neither the exact significance of each of its terms nor the form of the equation to be derived from the principle, particularly in relation to the integration of the function over time and the variability of the values which its terms can assume. He also did not analyze the conditions under which the equation was applicable: (*a*) establishment of a so-called stable state, (*b*) cyclic variations of alveolar ventilation not in phase with cyclic variations of blood flow into the lung capillaries, and (*c*) the phase relation between flow at the site of collection of samples and the rate of collection.

To the extensive use of cardiac catheterization as a valid means of measuring blood flow in man must be attributed the impetus given to proper formulation and validation of the principle so simply enunciated by Fick 80 years previously.

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[56] Zuntz, N., and Hagemann, O.: Untersuchungen über den Stoffwechsel des Pferdes bei Ruhe und Arbeit. *Landwirtschaftliche Jahrbücher, Zeitschrift für wissenschaftliche Landwirtschaft und Archiv der Königlich Preussischen Landes Ökonomie Collegiums*, XXVII Band, Ergänzungsband III, Paul Parey publisher, Berlin, 1898, pp. 1–438.

[57] Zuntz, N.: Eine neue Method zur Messung der circulierende Blutmenge und das Arbeit des Herzens. *Ebenda* LV: 1, 1893.

[58] *Journal de Physiologie* III: 695, 1860.

During the first quarter of this century cardiac catheterization suffered an eclipse from the physiologic scene The causes of the eclipse lay mainly in the use of artificial preparations for the study of the dynamics of the circulation, in particular the heart-lung preparation so brilliantly studied by Starling No one will deny the value of such studies They favor accurate measurements and well controlled experiments and they allow a careful analysis of the mechanical and physiological factors involved in a complex system But investigation in the intact animal has to my mind at least one superior merit namely, that it provides a more secure foundation for the application of physiologic thinking and techniques to the study of man. This application is the theme of the next episode in the history of cardiac catheterization.

I FIRST CONTROLLED CATHETERIZATION OF THE RIGHT ATRIUM IN MAN

e self-experiment reported by Werner Forssmann in 1929[*] provides a dramatic prelude to the era of cardiac catheterization in man. Forssmann was at the time 25 years of age. He had recently graduated from medical school and was pursuing his surgical training at the clinic of Eberswalde, a small town near Berlin. With the boldness of youth and against the advice of colleagues who had made an abortive prior attempt on him, he exposed one of his own left arm veins introduced a ureteral catheter into his venous system and advanced it, under fluoroscopic control and using a mirror up to his right atrium Forssmann allowed the catheter to remain in this position at least long enough that he could climb a few stairs to the X-ray department, where chest films were taken During the entire experience there were he reported no untoward effects due to the presence of the catheter (Figure 6)

According to a historical note by Benatt[*] this first successful controlled right atrial catheterization had been preceded by an experiment on a human cadaver in the course of which Forssmann realized how easy it was to guide a ureteral catheter up the arm vein into the right atrium One of his objects was to be able to use the technique as a means of rapidly administering undiluted emergency drugs directly into the heart. A first patient with purulent peritonitis was treated according to this procedure· Through the catheter she received 1 liter of glucose solution that contained adrenalin and strophantin the patient improved temporarily but then died At autopsy the tip of the catheter was situated near the opening of the inferior vena cava. Forssmann also cited as additional objectives the possible use of the method for study of the heart and for diagnosis which he did not further specify

Attempts have been made to disclaim Forssmann's priority In an addendum to his first report,[*] published a month later he acknowledged that Unger had informed him of earlier work by Bleichröder's group. Indeed in 1912 Bleichröder, Unger and Loeb reported several successful passages of a catheter into arm or leg veins of patients with subsequent advancement of its tip into the axilla, or into the inferior vena cava.[*] Some of these experiments were also made on themselves or their assistants. However these investigators did not control the exact location of the catheter tip by X-ray which probably was not available at this time Unger suggested however that during the course of an experiment in which Bleichröder was the subject he mentioned a stabbing pain in the

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[*] Forssmann, W Die Sondierung des Rechten Herzens. *Klin Wchnschr* 8 2085 1929

[*] Benatt, A J Cardiac catheterization. A historical note *Lancet* 746 1949

[*] In this addendum, published month later Forssmann quotes the letter he received from Professor E. Unger (*Klin Wchnschr* 8 2287 1929).

[*] Three separate notes were presented at a session of the Hufeland Medical Society in Berlin and reported (*Klin Wchnschr* 49 1503 191) Bleichröder in opening statement, recounted h experience with passing catheters into the arteries and eins of dogs and into the veins of man, back in 1905 these experiment were not published In his note of 191_, which deal with intraarterial therapy he mention that he had ntroduced catheters into the femoral eins of dogs mor than 100 times lea ng the probe / ider for several hours without observing any blood clotting or other ill effe

SONDERDRUCK AUS
KLINISCHE WOCHENSCHRIFT

DIE SONDIERUNG DES RECHTEN HERZENS
Von
Dr. Werner Forssmann

Fig. 6 Title page of the article describing Werner Forssmann's self-experiment. See text and note 59

chest, indicating that the tip of the catheter might have reached the right heart.

Other claims to priority do not even deserve mention.

In the light of what followed, Forssmann's experiments constituted remarkable progress. In one bold stroke he had demonstrated that a well known experimental technique, which he had applied under difficult but controlled conditions, could be extended to the study of man. This advance was potentially of paramount importance in the study of pathologic changes in the circulatory system, since such changes could be reproduced with difficulty or not at all in animal experiments.

During the next 2 years, Forssmann, first in Sauerbruch Surgical Clinic, where he found not support, but hostility, and then in Eberswalde again, studied in dogs a new method of radiologic examination of the right side of the heart, in which radiopaque contrast medium was injected directly through the cardiac catheter into the right atrium. These studies were reported in 1931 [63]. He also made at least six additional attempts to catheterize himself [64]. In these studies he injected 40% Uroselectan through the catheter without securing adequate X-ray visualization (Figure 7).

To conclude this all-too-brief account, the comments by Professor Liljestrand at the presentation of the Nobel Prize in Medicine or Physiology in 1956 on the motivation and on the unhappy experience of the protagonist of these studies in man seem fitting:

It must have required firm conviction of the value of the method to induce self-experimentation of the kind carried out by Forssmann. His later disappointment must have been all the more bitter. It is true that the method was adopted in a few places—in Prague and in Lisbon—but on the whole Forssmann was not given the necessary support. He was, on the contrary, subjected to criticism of such exaggerated severity that it robbed him of any inclination to continue. This criticism was based on an unsubstantiated belief in the danger of the intervention, thus affording proof that—even in our enlightened times—a valuable suggestion may remain unexploited on the grounds of a preconceived opinion. A contributory cause in this instance was presumably that Forssmann was working in a milieu that did not clearly grasp the great value of his idea. [65]

To be sure, the possible physiologic applications of Forssmann's experiments were rapidly realized.

Aus der II. chirurgischen Abteilung des Städt. Krankenhauses Berlin-Neukölln. (Prof. Dr. W. F. ...)

Ueber Kontrastdarstellung der Höhlen des lebenden rechten Herzens und der Lungenschlagader

Sonderdruck aus der Münchener medizinischen Wochenschrift 1931, Nr. 12, S. 489

(J. F. Lehmanns Verlag in München)

Fig. 7 Title page of the article by Forssmann reporting his experience with injection of a radiopaque substance through a catheter into the right atrium. See text and note 63

[63] Forssmann, W. Über Kontrastdarstellung der Höhlen des lebenden rechten Herzens und der Lungenschlagader. *Münch. Med. Wchnschr.* 78, 489, 1931. His presentation at a surgical meeting was the last one scheduled at the last session. It apparently did not arouse any interest in Germany for some time.

[64] These additional self-experiments were mentioned to me by Forssmann when we first met in Heidelberg, in November 1952.

[65] Göran Liljestrand. In *Le Prix Nobel en 1956*. Nobel Foundation, Stockholm, 1957.

In 1930 Jimenez Diaz and Cuenca described a successful attempt to introduce a ureteral catheter through a cannula into an arm vein of a moribund patient.[66] They were able to locate its tip in the right atrium and obtained X-ray evidence of its location They suggested many possible developments of this procedure therapeutic intracardiac injections in shock sampling of mixed venous blood for determination and comparison with peripheral venous blood contents of glucose lactic acid and respiratory gases injection of radiopaque substances for visualizing the pulmonary circulation and even measuring auricular pressure by connecting the catheter with a manometric system In a succeeding paper the same year [67] they reported studies on a few patients with cardiocirculatory insufficiency in which they compared the O content in peripheral veins and atrial blood and in one case the arteriovenous difference

There were no further publications on catheterization from Jimenez Diaz clinic In the same year however O Klein at the German University in Prague[68] reported the first measurements of cardiac output in several patients with various chronic systemic diseases some terminal the measurements were obtained according to the Fick principle, with mixed venous blood samples secured from the right atrium In 1932 Padilla Cossio and Berconsky of the University of Buenos Aires obtained mixed venous blood by the same method and by applying the Fick principle determined the cardiac output in two patients in order to compare the results with those obtained using the foreign gas (acetylene) method then in its early development.[69]

Apart from the immediate and rapid development of pulmonary angiography in clinical medicine in Portugal [70] Latin America [71] [72] and France [73] use of catheterization for study of the circulation in nor

---

[66] Jimenez Diaz, C and Sanchez Cuenca, B. El Sondage del Corazón Derechio *Archiva de Cardiologica y Hematologia* ol 11 March 1930

[67] Studios de Insufficiencia Circulatore. *Archivas de Cardiologia Hematologia* vol. 11 December 1930

[68] Zur Bestimmung de zirkulatorischen minuten Volumen nach dem Fickschen Prinzip (Gewinnung des schten emösen Blutes mittels Herzsondierung) *ench Med W knsch* 77 1311 Aug 1 1930 In this column report Klein refers to presentation at the November 1979 meeting of the Verein Deutscher Ärzte in Prague and another in April 1930 to the Wiesbaden Verh Dsche Ges. mer Medizin H gives many details regarding (1) the caliber of the ureteral catheter (he substituted a No 6 for the No 4 sed initially by Forssmann, so to avoid suction when sampling blood) (2) the site of introduction, a medial rather than an ternal vein, (3) the position of the arm during introduction of the catheter—aw *y from the thora to avoid entering the e ternal* jugular ein and (4) the discarding of the initial blood withdrawn, before sampling and transfer under oil of the blood collected I 60% of 18 attempts the tip of the catheter w located in the heart nder fluoroscopic control nine times the atri m once in the inferior ena cava, and twice in the right ventricle (without untoward effect). In three cases the Fick principle w applied to the measurement of cardiac output I two patient with normal circulations (one ith hronic di seminated sclerosis another with neuritis the output based on O_2 data w re 4 46 and 5 6 liters per minut I the third bject a patient with leukemia and anemia the cardiac output on the basi of both CO_2 and O data was measured respectively 6 93 and 6 6 lite per minute Klein did not appear to give attention to the stability of the metabolic state since not more than minutes lapsed between the introduction and the rid l of the catheter He reported no follow-up studie ugh during the ne t year he published several papers on vario medical subjects, including diabetes, histamine and pituitrin.

[69] These authors published four articles i succession on the subject of cardiac catheterization a first on technique (Sondeo del Corazón tecnica. *S mana Medica* 21 79 August 1932) a second on identification of the superior vena cava (Sondeo del Corazón. La vena cava superior y el borde derecho de la sombra de proyeccion de las grandes vasas de la base *Semana M dic* 2 391 Aug 11 1932) third on determination of cardiac output (Sondeo del Corazón: Determinación del Vol men minuto circulatorio *Semana Medic* 2:445 Aug 18 1932) and a fourth, in which they described different types of peripheral circulation and listed the possible applications of the technique of cardiac catheterization (Sondeo del Corazón. Diversa tipos de circulacion periferica *Sem na Medica* 645 Sept 8 1932)

These three physicians were part-time hosptans, pending thei mornings *in the Medical Clinic of the University* of Buenos Aires. The circumstances under which they pursued their clinical investigations were obviously not conducive to more systematic study and extension of the technique a tudy which could hav profited greatly from their close contact with Bernardo Houssay who wa then interested in the physiology of the lungs

[70] Ega Moniz Lopo d Carvalho, and Almeida Lima La visibilité des au seaux pulmonaires au rayons X pa injection dan l oreillette droit de fortes solution d'Iodure de sodium *Bull A ad de Med Pari* 105 627 April 14 1931

[71] Perez Ara A El Sondage del Corazón derechla su tecnica y aplicatione *Rev d Med Cir de la H bana* 36 491 J ly 31 1931

[72] Perez Ara. A Contribucion al estudio de la anomalia congentalias del Corazon *Rev de Med Cir de la Habana* 36 657 Sept 30 1931

[73] Ameuille O Ronneaux G Hinault, V Degrez and

mal and disease states was fragmentary and limited to exceptional instances which were of little importance in furthering physiologic or pathophysiologic knowledge. Obviously many possibilities were envisaged but no systematic plan nor the proper scientific background and facilities for its wide-scale implementation, were then available.

In short, the achievements of the early period of experimental studies were for the time being confined to a dead-letter office.

Lemoine, J. M.: Remarques sur quelques cas d'arteriographie pulmonaire chez l'homme vivant. *Bull. & Mem. Soc. Med. d'Hop. de Paris* 60:729, 1935. Severe criticism by the leading cardiologist in Paris was leveled at the authors, in spite of the fact that they had not encountered any mishaps. In the heated discussion which followed their presentation and the criticism, most participants favored discontinuing the procedure.

III THIRD PERIOD—1936–1945

The third of the periods I have recognized in the evolution of cardiac catheterization began quietly in 1936 The method was then still in eclipse in the experimental laboratory and its clinical use was sporadic and related to no definite programs of physiological inquiry Subsequent developments which here can merely be outlined provide what is to my mind a provocative illustration of the fertile interaction among a general plan of scientific inquiry technique contingency and a fortunate group of investigators.

I PRELUDE

In 1932 Dickinson Richards then at the Columbia-Presbyterian Medical Center and I chief resident of the Chest Service (Columbia University Division) at Bellevue Hospital in New York, agreed on a systematic and comprehensive examination of cardiopulmonary function, in normal and diseased man Our working hypothesis had been propounded by Lawrence Henderson The lungs heart and circulation form a single system for the exchange of respiratory gases between the atmosphere and the tissues of the organism [74]

In order to define the operation of this system (and thus validate the general concept) a number of methods were required Some of these methods were already available but others had to be devised "from scratch From the beginning we realized that implementation of our general plan presupposed methods for determining respiratory gas concentrations in mixed venous and arterial blood and for measuring cardiac output We succeeded in developing new techniques for studying various aspects of ventilatory and alveolo-respiratory function, but after several years we admitted failure of our attempts to equilibrate the CO_2 pressure in a rebreathing bag system with that in the mixed venous blood perfusing the lung capillaries In patients with lung disease inhomogeneity of alveolar gas distribution and capillary blood perfusion made the required equilibration unattainable [75] On the other hand insertion of a needle into a brachial artery could be accomplished routinely

In 1936 we considered whether the technique of right heart catheterization might provide help in obtaining the mixed venous blood needed for determination of respiratory gas concentrations In accordance with our general plan I arranged there fore to visit with Dr Ameuille one of my former teachers who had completed pulmonary angiography in 100 patients in his hospital in Paris [76] The protocols of these cases convinced me of the safety of the technique Dick Richards and I in collaboration with Robert Darling then familiarized ourselves with the method first in canine experiments and eventually in a chimpanzee [77] Meanwhile George Wright, a former student of Carl Wiggers and then a resident on the Chest Service at Bellevue Hospital offered to advance ureteral catheters under fluoroscopic control into the right atrium of human cadavers, in order to measure the distance between the catheter tip and a land mark on the anterior chest wall on lateral chest X-rays this datum would be needed to establish a reference point for measuring intracardiac pressures [78]

DEVELOPMENTS FROM 1940 TO 1945

In 1940 our first attempt on a patient failed because the catheter was obstructed at the level of the axil-

---

[74] For a succinct and comprehensive discussion of the development of this working hypothesis, see the text of the remarks made by D W Richards at the Respiration Dinner of the American Physiological Society at Chicago April 17 1957 (Lawrence Joseph Henderson *The Physiologist* vol. 1 1957)

[75] Richards, D W J Cournand A and Bryan N A Applicability of rebreathing method for determining mixed venous CO_2 in cases of chronic pulmonary disease *J Clin Invest* 14 173 1935

[76] *Op. cit* (note 73).

[77] The chimpanzee experiment remained unpublished until it appeared in the *P&S Quarterly* (17 15 Winter 1977) under the authorship of D W Richards who refreshed his recollection by referring to the experimental notebook of the Department of Pharmacology where the experiment had been performed.

[78] Unpublished data which served as the basis for determining the zero pressure level for subsequent pressure recordings using saline manometer

la.[79] Not long afterward however a new opportunity presented itself Homer Smith, Professor of Physiology at the New York University College of Medicine, with his collaborators including Drs. William Goldring, Herbert Chasis, and Hilmert Ranges at the Hypertension Clinic of Bellevue Hospital was planning to measure cardiac output in hypertensive patients using a new model of the ballistocardiograph[80] They had reached a stumbling block, however To calibrate the ballistocardiograph curves they needed reliable independent values of cardiac output determined at the same time as the ballistocardiograph recording. For this purpose, application of the direct Fick principle appealed to the brilliant physiologist, and there was little need for persuasion.

In a first report published in 1941 in collaboration with Ranges the results of initial experiences were presented The report emphasized (*a*) the necessity that the patient be metabolically stable (*b*) the requirement for simultaneous and prolonged blood and air sampling; (*c*) the value of the internal check provided by comparing results based on the application of the Fick principle to CO_2 and O_2 data (this resulted in rejection of at least half of the experiments) (*d*) the reproducibility of the results and (*e*) the acceptability to patients and innocuousness of the catheterization procedure[81]

The data on cardiac output were collected simultaneously according to the direct Fick and the ballistocardiograph procedures the latter data were interpreted using Starr s formula. Comparison of the results indicated that the ballistocardiograph method gave values lower by 30% on the average than those obtained with the direct Fick technique in the 32 patients tested[82] A few months later In 1941 Richards Cournand Darling and Gillespie published an initial report on simultaneous measurement of atrial and peripheral venous pressure with saline manometers in normal man and in patients with congestive heart failure[83] In 1942 an exhaustive report on the same subject included our experience in animals as well as in man.[84]

Between 1941 and 1945 several lines of technical advance were pursued

(*a*) A new catheter was designed. Its construction by maintaining a desirable balance between flexibility and rigidity assured ready maneuverability This probe was varnished with an unwettable radiopaque material and it had the minimal diameter compatible with facile sampling of blood using a slight degree of negative pressure Finally because of its curvature near the tip this instrument could be readily manipulated both intravenously and in the heart.[85]

(*b*) A cannula was also designed that facilitated insertion into the brachial or the femoral artery and prolonged cannulation of these vessels[86]

(*c*) With the assistance of Stanley Bradley our catheterization protocol was modified to include pressure recording with the Hamilton manometer[87]

(*d*) Later a special mobile multiple recording apparatus was designed in collaboration with Lauson and Richard Bloomfield It c electrocardiograph four fluid p

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[79] The attempt was made at the Presbyterian Hospital in early 1940 it was not reported. The obstruction was due to large lymph nodes in the presence of metastatic carcinoma.

[80] The new ballistocardiograph was designed by Dugald E. S. Brown of the Department of Physiology New York University College of Medicine. For description of this apparatus see the Appendix to subsequent report by Cournand et al (note 82).

[81] Cournand A., and Ranges, H. A., Catheterization of the right auricle in man. *Proc Soc Exper Biol & Med.* 46.462 1941

[82] The initial results were presented at the Annual Meeting of the American Physiological Society in April 1941 held in Chicago. For published presentation of the results, see Cournand, A. Ranges, H A. and Riley R. L., Comparison of results of the normal ballistocardiogram and direct Fick method in measuring the cardiac output in man. *J Clin Invest.* 21 287 1942. The average figure for cardiac output were using the Fick method, 6.23 liters per minute and using the ballistocardiograph method 4 49 liters per minute.

[83] Richards, D W J Cournand, A., Darling, R. C., and Gillespie W H. Pressure in the right auricle of man in normal subjects and in patients with congestive heart failure *Trans A Am. Phy* 56.218, 1941

[84] Richards, D W J Cournand A., Darling, R. C., Gillespie W H and Baldwin E. deF Pressure of blood in the right auricle, in animals and in man: Under normal conditions and in right heart failure. *Am. J Physiol* 134:115 1942.

[85] A line of catheters was manufactured according to our specifications by the United States Catheter Company Glen Falls, New York.

[86] This cannula was manufactured by Becton and Dickinson, East Rutherford New Jersey under the incorrect designation of "Cournand needle" the design of the needle was due to Richard L. Riley who followed suggestion by our research nurse

[87] Hamilton, W F., Brewer G and Brotman, I Pressure pulse contours in the intact animals. I. Analytical description of a new high-frequency hypodermic manometer with illustrative curves of simultaneous arterial and intracardiac pressure *Am J Physiol.* 107 4 4

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1944, 55, 34-36

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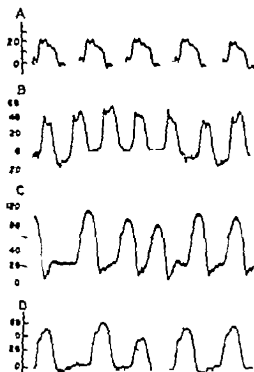
Recording of Right Heart Pressures in Man.

A. COURNAND, H. D. LAUSON, R. A. BLOOMFIELD, E. S. BREED, AND E. DE F. BALDWIN
(Introduced by W. W. Palmer.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University; of Physiology, Medicine and Surgery, New York University College of Medicine; and Bellevue Hospital, New York City.

Utilizing the right heart catheterization technic of Cournand and Ranges,[1] the cyclic pressure changes in the right auricle and right ventricle have been recorded with the Hamilton manometer[2] in 50 individuals.

Catheterization of the right auricle was performed as previously described.[1,3] The



1/5 0 2 SEC

FIG. 1.

All tracings were retouched to improve contrast in reproduction. Pressures in mm of mercury.

A. Right ventricular pressure pulses from normal young female. One respiratory cycle is shown.

B. Record from young male with extensive pulmonary fibrosis and normal sized heart. Cardiac output and arterial pressures normal. Note marked increase in systolic pressures; large respiratory variation due to dyspnea at rest; sharp drop of systolic and diastolic levels associated with beginning of inspiration; artifacts on systolic peaks.

C. Patient with mitral stenosis and insufficiency and aortic insufficiency and auricular fibrillation in congestive failure. Note extreme elevation of systolic pressures in right ventricle. The high diastolic pressures correspond to marked increase in right auricular and peripheral venous pressures. Note respiratory variation associated with dyspnea. Cardiac output subnormal and total blood volume about twice normal. Arterial pressure was 210/90.

D. Same patient after clinical improvement due to bed rest and digitalization.

patients experienced little or no discomfort during the procedure. The level of the catheter in the heart by lateral X-ray was taken as zero pressure. Introduction of the catheter with a slightly curved tip into the right ventricle under fluoroscopic control was not difficult as a rule and was signaled first by a rise above auricular pressure, then by large oscillations at cardiac rate in the saline manometer connected to the catheter.

Addition of the long narrow catheter (No. 8 or 9) to the manometer system decreases the natural frequency. Frequencies obtained with this system have varied from about 25 to 50 vibrations per second. Although not ideal, this range has proved fairly adequate

This investigation was carried on under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University with the collaboration of New York University. Additional support was provided by the Commonwealth Fund.

1 Cournand, A., and Ranges, H., PROC. SOC. EXP. BIOL. AND MED., 1941, 46, 462.

2 Hamilton, W. F., Brewer, G., and Brotman, I., *Am. J. Physiol.*, 1934, 107, 427.

3 Cournand, A., Riley, R. L., Breed, E. S., and Baldwin, E. de F., Interim Report, O.S.R.D. Contract OEMcmr 107, 1943.

Fig. 8. Title page of the article by Cournand, Lauson et al. describing the recording of right ventricular pressure in normal man and in patients with cardiac failure. See text and notes 89 and 90.

FIG. 9.
Simultaneous tracings in the right ventricle (upper) and in the pulmonary artery (lower) in a case of rheumatic valvulitis with hypertension in the lesser circulation. Note (a) the identity of form and pressure of both tracings during the later part of systole; (b) the small simultaneous oscillations on both tracings during early systole and diastole presumably due to motion of the catheter in the heart.

Fig. 9. The first pressure pulse tracings recorded in the pulmonary artery of a patient: from top to bottom, simultaneous tracings in the right ventricle and in the pulmonary artery. See text, legend and note 91.

manometers, a *fluid system for flushing*, a calibrating mercury manometer, and an optical system for transmitting deflections of the manometers without parallax to the recording camera; *the total length of* the optical bench was 6 feet.[90, 91]

These improvements in instrumentation were dictated by our concern for safety, efficient teamwork, adequate sampling of blood for analysis, and reliability of intracardiac and intravascular pressure tracings.

A different kind of fundamental advance resulted from our decision to advance the catheter tip into

[90] This mobile unit was constructed after discussion of, and improvement upon, our specifications by the Cambridge Instrument Company of New York City.

[91] The requirements for perfect recording of intracardiac pressures according to Otto Franck and Carl Wiggers were not achieved until many years later, when an electronic recording system was devised by Laurent in Paris. Unfortunately, in our early work we had not been able to interest electronic engineers in the design and fabrication of such a device. The tracings obtained through a catheter show artifacts of variable extent, as a result of the movement of the column of fluid, in turn a result of the beating of the heart. Moreover, the best natural frequency of the entire recording system never exceeded 40. But with adequate damping, tracings were obtained which correctly reflected systolic and diastolic pressures, integrated mean pressure, timing of initial deflections, and general form.

Fig. 10. Title page of an article describing a double lumen catheter (see Figure 9) which can be used simultaneously in adjacent right heart chambers or in the right ventricle and pulmonary artery. See note 92.

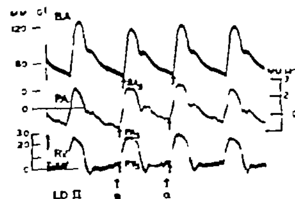


Fig 11 Simultaneous recordings of pulse pressures (from top to bottom) in femoral artery pulmonary artery and right entricle and electrocardiogram. These recordings were made in a man with normal circulation using the mobile multiple recording apparatus. See text and note 88

the right ventricle (Figure 8). This was done in 1942.[89-90] Two years later the catheter was advanced into the pulmonary artery (Figure 9) providing thereby the real key to studies of the pulmonary circulation in health and disease This last development and our repeated investigations of the problem of the optimal location for obtaining truly representative samples of mixed enous blood to construction of double lumen catheters (Fig e 10)[92] This made it possible to secure simultaneous blood samples and pressure recordings in the right atrium and ventricle (Figure 11) or in the right ventricle and pulmonary artery

During the same period i.e 194 -1944 we studied arious forms of shock, analyzing their physiologic mechanisms and evaluating the effects of different therapies Over a period of 4 years a *team of several individuals studied 103 cases in all* In each of these cases over at least a several-hour period cardiac output blood olume intracardiac and intraarterial blood pressures respiratory gas exchanges, blood gases and pH and in some cases, renal blood flow were repeatedly measured The results of this clinical in estigation were presented in a series of reports to th Committee on Medical Research of the Office of Emergency Management an arm of the Office of Scientific Research and Development, these res lt were also published in successive papers[93] and gi en elegant form n the Harvey Lecture of 1944 by D W Richards (Figure 12)[94]

Meanwhile, we also in stigated following similar protocol the circulatory effects of pressure breathing and phlebotomy in normal subjects and of bilateral sympathectomy in those with systemic hypertension. We also had the opportunity to study a patient with systemic hypertension before and *during the e olution of cardiac failure* Cardiac failure of two other types—that due to rheumatic heart disease and that associated with cor pulmonale—was compared on the basis of observations in respectively seven and six subjects. These studies were presented at a symposium on cardiac output, organized by the American Physiological Society in Philadelphia in 1944 and published in the *Federation Proceedings*[95] in 1945 (Figures 13–17)

During this period original contributions marking

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[89] Semi-annual report of work under Contract 107 with O E.M (C M.R.) for the period from September 1942 to March 1943 see pp 35 58 and chart 13 (National Research Council, Washington, D C.).

[90] Cournand A., Lauson, H. D., Bloomfield R. A Breed E. S., and Baldwin, E. deF Recording of right heart pressures in man. *Proc Soc Exper Biol. & Med* 55 34 1944

[91] The first recorded pressure tracings in the pulmonary artery of patient with rheumatic heart disease in failure figure in report published in *Proc Soc Exper Biol & Med* 1945 (note 97)

[92] Cournand A Bloomfield R. A., and Lauson, H D Double lumen catheter for intravenous and intracardiac blood sampling and pressure recording. *Proc Soc Exper Biol & Med* 60 73 1945

[93] See Cournand, A., Riley R. L. Bradley S E., Breed, E. S Noble R P Lauson H D Gregersen M. I and Richards, D W Studies of the circulation in clinical shock. *Surgery* 13 964 1943 Cournand A Studies of the circulation in shock. *Proc Rudolf Virchow Med Soc* .. 151 1943 Lauson, H D Bradley S E and Cournand, A The renal circulation in shock. *J Clin Invest* 23 381 1944 Cournand A., Noble, R. P., Breed E S Lauson, H D Baldwin, E. deF., Pinchot, G B., and Richards, D W J Chemical, clinical and immunological studies on the products of human plasma fractionation VIII Clinical use of concentrated human serum albumin in shock, and comparison with whole blood and w th rapid saline inf sion. *J Clin. Invest* 23 491 1944 and Richards, D W J., and Cournand A., Circulation in shock. Mechanical and vasomotor factors. *Trans A Am Phy* 58 111 1944

[94] The Circulation in Traumatic Shock In Man. *The Harvey Lecture Series* XXXIX, 217 .53 1943–1944 Chapter XXVII of *Advanc Military Medicine* (Little Brown, nd Co Boston, 1948), which reported all the project completed under the auspices of O E.M (C M R) during World War II contains section on the clinical aspects of shock which wa written by D W Richards (pp 335–341)

[95] Cournand A Chairman: Symposium on cardiac output *Fed Proc* 4 183 1945

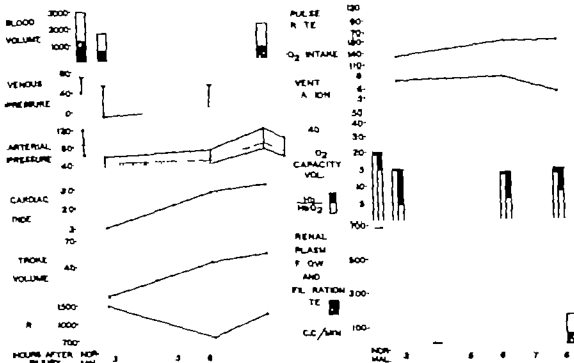


Fig 12 Illustration showing all data obtained over an 8-hour period from a patient with traumatic shock On the horizontal time scale the solid bar represents the duration of blood transfusion For further details see text and note 94

the extension of cardiac catheterization into new fields were made by former collaborators or by investigators trained in our laboratory Stanley Bradley a member of our initial team after his transfer to Boston, introduced as an investigative tool catheterization of the renal[96] and hepatic[97] veins James Warren in Atlanta,[98] and Eleanor Baldwin and Robert Noble[99] at the Columbia-

[96] Catheterization of the renal vein with an extra-length (four-foot) radiopaque catheter by Bradley and Curry is described in Appendix G (p 219) of *Hypertension and Hypertension Disease* by William Goldring and Herbert Chasis (The Commonwealth Fund New York, 1944) The authors indicated that J V Warren, A J Merrill and E S Brannon had been engaged in similar studies to determine the percentage withdrawal of sodium para-amino hippurate

[97] Bradley S E Ingelfinger F J Bradley G P and Curry J J The estimation of hepatic blood flow in man. *J Clin Invest* 24 390–397 1945

[98] Brannon, E S Weens, H. S and Warren, J V *Atrial septal defect* Study of hemodynamics by the technique of right heart catheterization *Am. J M Sc* 210 480 1945

[99] Baldwin E. deF Moore L. V and Noble, R. P The demonstration of ventricular septal defect by means of right heart catheterization *Am. Heart J* 32: 152 1946

TABLE 1

Effect on cardiac output of continuous positive pressure breathing (20 mm Hg) in four normal subjects at sea level

Subject	Breathing pressure	Oxygen uptake	Cardiac index
	mm. Hg	cc./min.	liters
L. K.	0	[illegible]	[illegible]
	+20	[illegible]	[illegible]
	0	[illegible]	[illegible]
G T	0	[illegible]	[illegible]
	+20	[illegible]	[illegible]
	0	[illegible]	[illegible]
G G	0	[illegible]	[illegible]
	+20	(107)	[illegible]
	0	[illegible]	[illegible]
E K.	0	[illegible]	[illegible]
	+20	[illegible]	[illegible]
	0	134	[illegible]

Intervals between successive measurements about 30 minutes.

Fig 13–17 Tables published in *Federation Proceedings*, 1945 See text and note 95

Fig 13 Effects on cardiac output of continuous pressure breathing

vestigators and none other to my knowledge has had the auspices of a perfect and lasting collaboration between two investigators with complementary qualities

In retrospect it would appear that the following were among the considerations which helped to make possible the successful extension to man of the technique pioneered by Claude Bernard (*a*) The aim was always to improve diagnostic methods and to establish treatment of disease processes on a rational basis. (*b*) The means included an assemblage of valid techniques, used with awareness of past and present physiological developments (*c*) Colleagues were inspired with confidence as to the validity and reliability of the results and in the patients under our care as to the safety of the method (*d*) We were able to take advantage of contingencies and to eliminate unnecessary risks (*e*) Strict discipline was enforced in applying the technique in developing efficient team work, and in analyzing physiologic and pathologic conditions

In a word we were fortunate enough to achieve in some measure the fundamental association of experimental and clinical medicine of which Claude Bernard dreamed

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Studies in asymptomatic primary hyperlipidaemia

I Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations

By Anders G Olsson and Lars A. Carlson

cal Company Stockholm, Sweden

Acta Medica Scandinavica

originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD In 1901 (from volume 34) this journal was divided into a medicial and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*

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Studies in Asymptomatic Primary Hyperlipidaemia

I Types of hyperlipoproteinaemias and serum lipoprotein concentrations, compositions and interrelations

By
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CONTENTS

Abbreviations	IHD = ischaemic heart disease
	HL = hyperlipidaemia
	LP = lipoprotein
	HLP = hyperlipoproteinaemia
	TG = triglycerides
	VLDL = very low density LP
	LDL = low density LP
	HDL = high density LP

INTRODUCTION

Atherosclerosis is chronic disease developing over decades. Already during childhood and the first decades of life raised atherosclerotic lesions have been found at autopsy studies (53). The sudden clinical manifestations of atherosclerosis, such as myocardial infarction as well as those more slowly developing such as intermittent claudication, are merely the final clinical consequence of this long-lasting pathological process of the vessel wall.

Epidemiological studies during the 1950 ies and 1960 ies have revealed numbe of "risk factors for clinical manifestations of atherosclerosis, for instance ischaemic heart disease (IHD) The presence of such risk factors in an individual is associated with an increased risk for the development of IHD. Important risk fact rs for IHD are hyperlipidaemia (HL) glucose intolerance cigarette smoking and hypertension.

With regard to HL retrospective (3 11 42) and prospective (16 30 54) studies have demonstrated that elevation f serum cholesterol (30 42, 54) and triglyceride (TG) (3 11 16) concentrations are of importance in the devel pment of IHD. The serum cholesterol and TG are contained in three different majo lipoprotein (LP) classes but only scant information on the role played by these different LPs in the development of IHD is available. Considering the LPs in HL, Fredrickson et al (24) introduced a syst m which in its modified form (4) describes si different types of hyperlipoproteinaemia (HLP) The importance of these types f HLP in therosclerosis and its manifestations is not well defined. Retrospective studies have shown that elevation of both very low density LP (VLDL) and low density LP (LDL) (types IV II A nd II B HLP) as w ll as decreases f high density LP (HDL) (11 17B 40) are associated with IHD but prospective studies on th role of the different types of HLP in the development of IHD are lacking. Retrospective as well as prospective studies ha several disadvantages in studying the relation between HLP nd IHD. In retrospective studies the clinical event in itself might have influenced the LP concentrations as well as oth r risk factors. Also many subjects have died during the acute phase of IHD. Prospective studies on the other hand are time consuming, require large numbers of subjects and would be quit costly if qua t tative LP analyses were to be performed.

Against this background we considered t of practical and theoretical value to investigate newly discovered hyperlipidaemic but otherwise healthy subjects for the prevalence of atherosclerosis in the coronary arteries and the art ries of the lower limb in relation to differe t types of HLP It is not possible today to estimate the degree of atherosclerosis by non-invasive techniques. Therefore w have chosen to investigate persons with HLP using methods which might disclose ischaemia and changes in arterial flow induced by atherosclerosis, the results serving as probable early signs of atherosclerosis. Furthermore by se rching for metabolic abnormalities in such asymptomatic subjects in whom arterial disease has not yet given rise to symptoms, etiological mechanisms behind the HLP might be bett r elucidated than by st dying patients with HLP and ymptoms of therosclerosis.

We have called the concept of tracing subclinical signs of a disease process in the presence of a certain characteristic – e. g. a risk factor such as HLP – before the appearance of the potential linical event an *interspective study"* The early detection of HLP also enabled us to take primary preventi measures i. e to try to interrupt the process of atherosclerotic disease We ha under taken an interspective st dy in order to describe the presence of probable early signs of therosclerosis in different types of HLP The occurrence of these signs has also been determined in a control group with non-elevated concentrations of serum cholesterol and TG. HL has been defined in terms of LP concentrations and the HL hence is called HLP

The purpose of this paper is to describe first the

serum lipids of the sample from which the HLP subjects were drawn ("The Metropol sample") and the formation of the interspective HLP and control groups. After that the frequency of different types of HLP and the LP concentrations, compositions and interrelationships in HLP will be reported.

'THE METROPOL SAMPLE"

PROCEDURE AND METHODS

"The M tropol Health Control Company" (Hälsokontrollen Metropol) Stockholm (Head Sture Helander M. D) in the following called Metropol, annually performs regular health examinations of around 15 000 people living in the Stockholm metropolitan area. The subjects coming to Metropol are employees of around 400 different companies which offer the health examination annually fre of charge. The examination is oluntary and takes place during working hours. The result of the examination is only available to the subject and the doctor responsible. Inquiry was made to the taff manager of 25 randomly selected companies utilizing M tropol regarding the participation rate This was 95± 2 (SEM) per cent (range 75–100 per cent). *At Metropol, all subjects gave their* medical history and received a complet physical examination by a physician. A resting ECG was recorded. Urine was analysed for the presence of glucose and protein and bacteria (BM test 4 Boehringer-Mannheim) Haemoglobin and ESR were also determined. Blood was taken every third yea for serum cholesterol (6) and TG (31) analyses by venipuncture in the sitting position with as light venostasis as possible. For practical reasons blood sampling had to take place also in the fternoon and therefore both fasting and non-fasting subjects were xamined. Clotting proceeded t room temperature for 1– hours, serum was recovered after slow speed centrifugation and stored t –20° C. The samples from one week, usually arou d 150 were analysed the next week at K ng Gustaf V Research Institute on one week day by the same technicians throughout the study on an Autoanalyzer Model 1 (Technicon Corporation) Cholesterol and triolein (reagent grade) from Sigma Chemicals were used as standards. Two control standard sera with defined and different concentrations (deep frozen pooled samples, not older than 6 months) were included in all runs. All determinations of unknown samples were corrected according to the deviation of the standard serum. Standard sera were not allowed to deviate from each other by more than 3 per cent w thin each run. The error f the methods of the determination of cholesterol and TG have been around 4 and 5 per cent respectively

When not otherwise stated, statistical treatment was performed according to Snedecor (52).

RESULTS

Serum lipids were analysed in 20 13 samples from 11 898 males and 8 234 females from 77 October 1970 till 18 March, 1974

The distributions of serum cholesterol and TG of all men and women aged 41–45 years are given in Figures 1 and and T ble 1 to exemplify the "Metropol lipid pattern" The distribution curves clearly demonstrate the difference between the response of serum cholesterol and TG to the non-fasting state. In both sexes, the approximately normal distribution of the cholesterol concentrations were very similar in the fasting and non-fasting states. Mean values did not differ in men, but non-fasting women had total serum cholesterol slightly higher than fasting ($p < 0.05$).

On the other hand the skewed distribution of serum TG (10) was still more pronounced in the non-fasting state. Both the distribution curves of the TG values themselves and of their logarithms were displaced to the right (Fig. 1 and 2) in non-fasters and the mean serum TG was increased by around 50 and 30 per cent in men and women in non-fasting as compared to fasting state.

Although the distribution curves of total serum cholesterol in the two sexes were similar and approximately normal, the mean value in fasting men was 3 per cent higher ($p < 0.005$) than in fasting women. No difference was seen in mean

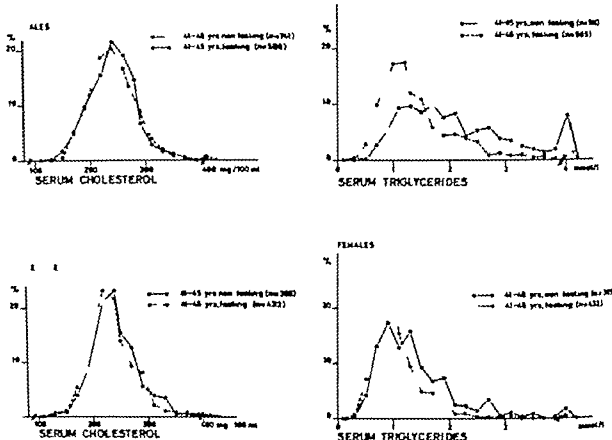


Fig. 1 Serum cholesterol and TG in non-fasting and fasting males and females aged 41–45 years in the M tropol sample.

Table I Mean fasting and non-fasting serum cholesterol and TG concentrations in 41–45 year old males and females t Metropol

	MEN		WOMEN	
	Fasting 505	Non-fasting 714	Fasting 432	Non-fasting 365
Cholesterol, mg/100 ml				
Mean	239	238	232	239
SE	2	1	2	2
% > 340 mg/100 ml	1 8	1.6	1.6	1.6
% > 400 mg/100 ml	.6	1	.2	.2
TG mmol/l				
Mean	1.50	2 24	1 07	1 43
SE	0.04	0.05	.03	.06
% > 3.40 mmol/l	3 8	13.0	1.2	2.7
% > 4 00 mmol/l	2.3	8 1	1 2	1.6
Mean[1]	1 31	1 94	97	1 24

[1] Calculated on logarithmic values and reconverted.

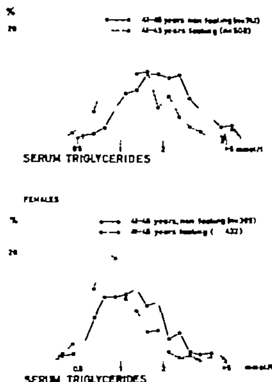


Fig. 2. Log TG in non-fasting and fasting males and females aged 41–45 years in the Metropol sample.

serum cholesterol concentration between non-fasting men and women. The proport on of men and women with fasting serum cholesterol above 340 mg/100 ml was of similar magnitude. Mean serum TG concentration was 40 percent higher in fasting males tha in females. The difference between the sexes is also demonstrated by the fact that three times more men than women had fasting TG concentrations above 4 mmol/l.

DISCUSSION

As seen before in this city (18) and elsewhere (e. g. 2) males and females 41—45 years have similar serum cholesterol concentrations while that of fast ng serum TG is higher in males (18). In the non-fasting tat the same sex relations existed for the serum lipids. The non-fasting state had exerted little or no influence on cholesterol values while the TG had increased considerably. For a long time it has been a well known fact that alimentary lipaemia gives rise to higher serum TG concentrations. If the increase in serum TG of 0.7 mmol/l or 60 mg/100 ml was due only to chylomicrons it can be calculat d from the known composition of this LP class that the cholesterol cont t of these chyl microns would be about 1–2 mg/100 ml, value which is of similar magnitude as th observed differences in total serum cholesterol between fasters and non-fasters.

It is concluded that the state of fasting of subjects in lipid screening on a health control basis is of no practical importance as far as the serum cholesterol concentration is concerned. However as serum TG is profoundly altered in the non-fasting state non-fasting individuals displaying hypertriglyceridaemia should be re-investigated in the fasting state.

Ten years before the present study serum lipids were analysed at another health control ce tre in Stockholm (18). In this study The Stockholm Prospective Study the mean values (± SEM) were for holesterol (Sperry Webb method equivalent see ref. 18 p. 4) in males and females 40–44 years 57 ± 3 and 48 ± 3 mg/100 ml. The corresponding figures for fasting serum TG were 1 58 ± 0.04 and 1 07 ± 0.03 mmol/l. In th present study mean serum cholesterol values were thus 10–20 mg/100 ml lower than in the Stockholm Prospec tive Study while serum TG were almost identical. The percentage number of 40–45 year old males having serum cholesterol concentrations above 340 mg/100 ml in Stockholm prospective (n=289) and interspective (n=505) study were 2.7 and 1.8 respectively For women th corresponding figures were 3 0 (n=269) and 1 6 pe cent (n=432) respec tively The diff rences were not significant (22). In the same age group th percentage fasting serum TG of 3 00 mmol/l and bove in Stockholm prospective and Interspective studies were 5 5 and 5 7 per cent (n. s.) in men and 0 4 and 1 6 per cent (n. s.) in women.

It should be kept in mind that these two studies were not designed for th comparison of serum lipid values in 1961/62 and 1970–1974 The two different cholesterol methods have, however been repeatedly checked and standardized against the Sperry-W bb method at King Gustaf V Research Institute and supervised by one of us

(L.A.C.) The cholesterol method used in this study gives close to identical values with the Sperry Webb method, while the values from the prospective study can be transformed by a regression equation to be nearly identical to Sperry Webb (18) It is thus not unlikely that the lower mean cholesterol values observed in this study reflect a true decrease in serum cholesterol in subjects attending health control centres linked to their employment. The reason for this may at least partly be changed dietary habits. The total amount of fat available for consumption per capita in Sweden decreased between 1965 and 1973 from 123 g to 118 g daily (35). The percentage fat calories in the food decreased from 40.4 to 38.6 per cent. On the other hand the consumption of linoleic acid increased both in relative and absolute terms. One possible explanation to the observed decrease in serum cholesterol may therefore be that the consumption of saturated fats has decreased and that of polyunsaturated fats has increased during the last decade.

The method for serum TG used here agrees well with Carlson s method (12) which was used in the Stockholm Prospective Study Therefore we believe that there have not been any significant changes in the serum TG levels since 1961/62.

FORMATION OF THE INTERSPECTIVE SAMPLE

MATERIAL AND METHODS

Procedure

The screened subjects were grouped into different categories regarding th serum cholesterol and TG concentrations. Those with serum cholesterol *above approximately 350 mg/100 ml and fasting* serum TG above 3 5 mmol/l were with the exceptions noted below referred to the Lipid Unit at the Department of Internal Medicine Karolinska Sjukhuset for further examination and treatment. If in a n n-fasting subject it was unclear whether an elevated serum TG value was due t alimentary lipa mia only the subject was called back for a fasting sample at Metropol. The above procedure was then pplied.

At the first visit t the Lipid Unit, fasting blood was taken for serum lip d determination and LP analysis The interval between the fasting blood samples t Metropol and th Lipid Unit was *107 ± 3 (SEM) days. The same procedure was* followed for the blood samples from the Lipid Unit as fo th samples from Metropol with regard to serum lipid analysis. Other laboratory tests performed were haemogl bin, white blood cells count erythrocyt sedimentation rat (ESR Westergren method) serum transaminases, alkalin phosphatases, serum creatinine, serum uri acid, fasting blood glucose PBI and T3 resin test, and serum electrolytes. Urine was analysed for th presence of protein and glucose All these tests were made by clinical routine methods in use at the hospital. At the same visit body weight was measured without overcoat and jack t. Half a kg was subtracted from the observed weight as an arbitrary weight for shoes and clothing. Body height was measured without shoes. All subjects were int rviewed and examined by one of us (A.G.O) A standardized clinical routine with a special medical record djusted to be computerised was used.

Interview

Case history involved family history and questions were always asked concerning the known presence of HLP atherosclerotic manifestations, diabetes and thyroid diseases within the family

Smoking habits, alcohol consumption and preceding *illness were recorded Direct questions were* asked regarding the following diagnosis or symtoms myocardial infarction, angina pectoris, congestive heart failure cardiac arrythmias, systemic hypertension, cerebrovascular disease, intermittent claudication, diabetes gout, liver disease or other major diseases such as pulmonary embolism and malignant disease. For chest pain on effort and intermittent claudication the Rose and Blackburn questionnaire (48) was essentially followed.

Questions were also asked as t current chronic drug treatme t.

The physical examination

The physical examination included clinical routine examinations, a search for stigmata of HLP (corneal arcus, xanthelasmat and tendon and skin xanthomat) and for signs of hypothyroidism.

Cardiovascular examination. The cardiovascular examination included auscultation of the heart bdominal aorta, carotid, subclavian and femoral arteries. Blood pressure was measured in the right arm with a mercury manometer after five minutes t rest in the supine position. The cuff was 13 5 cm wide. Readings were recorded at the nearest 5th mm Hg. The diastolic blood pressure was recorded at the disappearance f the 5th Korot kow sound.

Exclusions

Patients with previous diagnoses or present symtoms of atherosclerotic diseases were excluded. The following diagnoses were found (numbers

within brackets) myocardial infarction (4) angina pectoris (3) congestive heart failure (1) cerebrovascular diseases (4) intermittent claudication (3) abdominal aortic aneurysm (1) and amaurosis fugax (1) Diagnosis was based on the history of the patient notes in the medical records of Metropol and on hospital medical records

Subjects with disorders that could influence the interpretations of ECG recordings and peripheral circulatory investigation were also excluded. Diagnoses in this category were systemic hypertension (23) and cardiac arrythmias (2) The definition of hypertension followed the critera of Master et al (37).

In order to exclude secondary HL the following exclusions were made (number within brackets) history of or present diagnosis of dysfunction of the thyroid gland (18) diabetes (2) gout (4) liver (2) or renal (1) disease Patients with history of malignant disease (3) were all excluded. Patients treated with any of following drugs were excluded hypolipidaemic compounds (2) cardiac glycosides (5) diuretics (18) antihypertensives (3) adrenergic β recepto blocking agents (11) vasodilators (3) and hormones or agents with hormonal action including peroral contraceptives (13) Subjects were often excluded for more than one of the reasons mentioned. Subjects were not excluded because of obesity

Other investigations

After the first visit to the Lipid Unit the subjects were referred for other investigations such as intravenous glucose tolerance test intravenous fat tolerance test exercise ECG chest x-ray and studies of the peripheral circulation. After these studies treatment of the HLP was begun.

The control groups

The control group was chosen to comprise subjects attending Metropol having non-elevated serum cholesterol and TG concentrations. On at least two occasions they should have had fasting TG and cholesterol concentrations below 2.00 mmol/l or 300 mg/100 ml respectively Two hundred and twenty-two subjects with serum lipid concentrations below these limits were offered identical investigations as the HLP subjects. Controls were age and sex matched to the HLP group. Exclusions were made according to the same criteria as in the HLP group. Twenty five subjects were excluded at the Lipid Unit because of diagnosis or drug treatment Encountered diagnoses or chronic drug treatments were cardiac valvular disease (1) cardiac arrythmia (1) systemic hypertension (1) liver disease (1) kidney disease (6) thyroid disease (4) tuberculosis (4) malignant disease (3) diuretics (3) and estrogens (1) Of those offered examination 51 subjects (23 per cent) did not wish to participate 14 (6 per cent) did not reply and 4 (2 per cent) had moved away

RESULTS

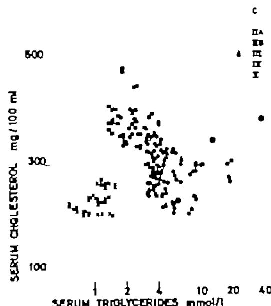
The interspective sample

During the period of study 705 subjects exhibited "marked HL of whom 314 HL subjects, 188 men and 126 women fulfilled the criteria and participated in the interspective study The formation of this "interspective sample" is detailed in Table II. After a certain time a reasonable number of males with type IV and females with type II A had accumulated while other types were few At that stage because of capacity problems, subjects (n=176) with these frequent HLPs were not called to the interspective study Of the 420 subjects called to the interspective study 7 per cent did not wish to participate and 16 per cent were excluded at the Lipid Unit.

Table II Formation of the interspective sample. Number of subjects

Screened sample			20 132
HL			705
HL excluded at Metropol because of diagnosis	109		
HL not called to interspective study[1]	176		
Total HL excluded from interspective study		285	
HL called t interspective study			420
Not interested	30		
Moved away	5		
Dead	2		
Excluded at the Lipid Unit	69		
All excl sions t Lipid Unit		106	
INTERSPLCTIVE SAMPLE			314

[1] See text.



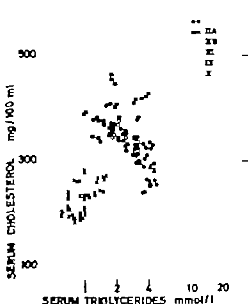


Fig. 3. Initial serum lipid concentrations in males in the interspective sample. C = control group. N subjects with normal LP type. II A, II B, III, IV and V indicate types of HLP (4).

Fig. 4. Initial serum lipid concentrations in females in the interspective sample. See legend to Fig. 3.

The serum lipid values

Initial holesterol and TG concentrations for men and women in HLP and control groups are given in Figures 3 and 4. High TG values were more abundant among males than females, while more females had high cholesterol values.

The difference between th first and second valu for the serum lipids

The first and second serum cholesterol concentrations for the fasting subjects are given in Figure 5. The cholesterol values tended to be lower at the second determination. This tendency was m re pronounced the higher the values. The number of males with higher/lower values for cholesterol at the second determination was in the cholesterol region below 300 mg/100 ml for the first deter mination 9/79 in the regio 301–400 mg/100 ml 12/66 and for cholesterol above 400 mg/100 ml 1/13. The corresponding figure for females were 9/7 21/53 and 1/11.

The plots for the first and second serum TG concentrations are given in Figure 5. There was a tendency also for lower serum TG concentrations at the second estimatio that at the first and this tendency was greater the higher the initial serum TG. If the initial serum TG concentrations were divided into the regions below 2.00, 2.01 to 4.00 and above 4.00 mmol/l the number of males with higher/lower values for TG at the second determination was 19/6 19/45 and 15/45. The corresponding figures for f males were 21/14 22/30 nd 5/10.

DISCUSSION

The results of the procedure of serum cholesterol and TG screening of the Metropol sample show that it is possible to pick out subjects with primary HLP who have not experienced any symtoms of atherosclerotic disease.

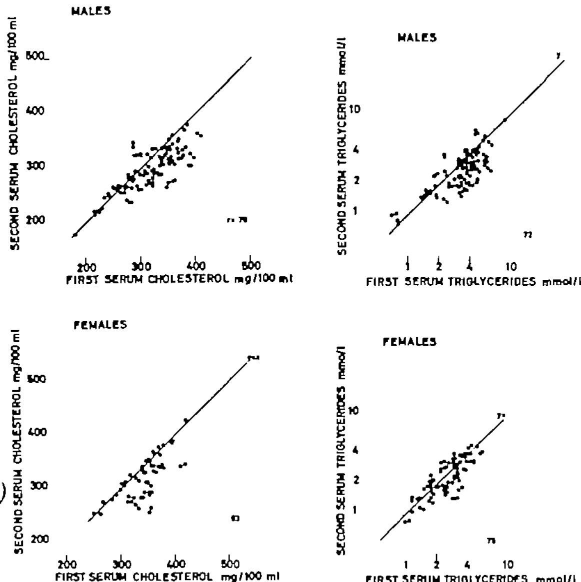


Fig. 5. Second (LP analysis) in relation to first (screening) fasting serum cholesterol and TG concentrations in males and females in the interspective sample.

This study did not aim at describing a "randomly selected" Stockholm population but to describe the conditions in a health control sample" This sample is of great interest as it constitutes those subjects available fo active preventive medicine.

The highly selective mechanism of the different stages involved to obtain this HLP group makes it impossible to draw any conclusions as to the prevalence of different types of HLP in Stockholm. The significant correlation between th first and second serum cholesterol and TG concentrations suggests that most of these values reflect the habitual serum lipid concentrations of the subject. There was a fairly large variation between the first and second serum lipid value and a clear tendency towards lower values for both serum lipids at the second determination. Several mechanisms explain these facts. Both serum cholesterol and TG normally fluctuate and thus the phenomenon of "regression to the mean" will operate when sub-

jects are recruited by selecting those with th highest values. Also all subjects were informed at Metropol of their serum lipid concentrations and whether or not this concentration was elevated. Some subjects therefore might have changed their dietary habits as the population in Stockholm in general is well informed concerning the relation between diet and HLP

FREQUENCY OF TYPES OF HYPERLIPOPROTEINAEMIAS AND SERUM LIPOPROTEIN CONCENTRATIONS, COMPOSITIONS AND INTERRELATIONS

LP analysis and typing of HLP was performed on sera of all HL subjects participating in the inter spective study

METHODS FOR LIPOPROTEIN DETERMINATION

LP separations were made by preparative ultracentrifugat on and paper electrophoresis. Serum was inspected after standing overnight at 4 C as a qualitative test for chylomicrons (4) The procedure of the preparative ultracentrifugation has been previously described in detail (9) nd involves in essence the separation of the three LP classes normally present in the fasting state very low density LP (VLDL) which corresponds to pre-β on electrophoresis, low density LP (LDL) corresponding to β-LP and high density LP (HDL) with α mobility on electrophoresis. After the first centrifugation at d=1 006 the t p layer recovered from the centrifugation tube contains VLDL. The bottom fraction containing LDL and HDL is then centrifuged at d 1 063 and LDL nd HDL are recovered in the top and bott m fraction of the tube respectively To increase the density a solution of NaCl-NaB is added. The three separated fractions VLDL, LDL and HDL are then xtracted by isopropanol for determination of cholesterol (6) and TG (31) concentrations. In the LDL and HDL fractions bromide was removed from the isopropanol by the addition of 0.2 g Dowex 1–x8 (Cl) per ml. The results of the LP determination were ccepted f r regressions and correlations only if the recovery of cholesterol and TG in the three LP classes was 87–113 per ce t of the total lipid concentrations. The average recovery in each of the six types of HLP varied between 93–99 per cent. For typing, lower or higher recoveries could be accepted if the lacking or excess LP lipid could not affect typing.

LP paper electrophoresis was performed according to Lees and Hatch (34) on whole serum, and on top and bottom fractions after separation in the ultracentrifuge at d=1 006 Three bands pre-β β and α LP bands were visualized. Electrophoresis on the top and bottom fraction after separation at d=1 006 was performed in order t detect "floating-β" LP (see below).

TYPING OF HYPERLIPOPROTEINAEMIAS

Typing of the HLP serum was performed according to the principles of Fredrickson et al. (24) as modified in a WHO memorandum (4) Upper normal limits were based upon a normal adult male material in Stockholm where the LPs were determined by the same method as used here (15) Arbitrarily the upper limit was in rounded off figures set at the value fo mean plus twice the standard deviation. The upper limits used were for LDL cholesterol 220 mg/100 ml and for VLDL TG 1.25 mmol/l. It is recognized that there are age and sex differences for the LP concentrations (17). We have deliberately only used the above values for all subjects, aiming at obtaining similar LP concentrations for the two sexes and all ages in the different types of HLP The following criteria for classification into the different types of HLP were used.

Type II A HLP

1 LDL cholesterol concentration of 220 mg/100 ml or more.
2. VLDL TG concentration below 1 25 mmol/l.

Type II B HLP

1 LDL cholesterol concentration of 220 mg/100

ml or more
2. VLDL TG concentration of 1 25 mmol/l or more
3 Presence of a pre-β LP and absence of a β-LP in the isolated VLDL on paper electrophoresis.

Type III HLP
1 Presence of "floating β" LP or "β-VLDL" visible as a band with β mobility on paper electrophoresis of the isolated VLDL.
2. VLDL TG concentration of 1 25 mmol/l or more.

Type IV HLP
1 VLDL TG concentration of 1 25 mmol/l or more.
2. LDL cholesterol concentration below 220 ml/100 ml.
3 Presence of a pre-β LP and absence of a β-LP in the isolated VLDL on paper electrophoresis.

Type V HLP
1 2 and 3 as in type IV HLP
4 Fasting chylomicronaemia

"Normal (N) type
1 VLDL TG concentration below 1 25 mmol/l.
2. LDL cholesterol concentration below 220 mg/100 ml.

The control group

In the control group, total serum cholesterol and TG estimation together with LP paper electrophoresis on whole serum was performed at the first visit. For practical reasons LP separation with preparative ultracentrifugation could not be made concomitantly with the other investigations of the controls. This was performed instead during the summer of 1974 Of the male and female controls, 78 and 88 per cent respectively returned for serum LP determination. The average time that had elapsed between the determination of total serum lipids and LP concentrations was 23 months. Mean body weight, mean total serum cholesterol and TG had not changed significantly in either sex during this period.

RESULTS

Frequencies of the different types of hyperlipoproteinaemia

In the interspective sample, the percentage frequencies of the different HLP types were (males/females) normal pattern (N) 19/25 type II A 16/37 II B 7/10 III 6/5 IV 50/24 and V 2/1 Type IV was thus by far the most common HLP type in males. N and type II A were of equal prevalence being about one-third as frequent as type IV Type II B and type III were less common and type V was rare. In women the most frequent HLP type was II A while type IV was less abundant. The relative frequency of the other HLP types was of the same order as in men

As stated above the choice of cut off points between "normal" and elevated" levels is arbitrary The effect of changing the cut off points for VLDL TG and LDL cholesterol on the allocation

Table III The effect of the use of different cut off points for defining elevated VLDL TG and LDL cholesterol on the distribution of the HLP subjects on different types. Number of subjects

	MEN			WOMEN		
VLDL TG mmol/l	1.10	1.25[1]	1 40	1 10	1.25[1]	1 40
LDL cholesterol mg/100 ml	200	220	240	200	220	240
N[2]	22	35	49	16	31	49
II A[3]	31	31	26	53	46	33
II B	40	14	3	27	12	8
IV	81	94	96	23	30	29
V	3	3	3	1	1	1

[1] Cut off points used for classification in this study
[2] N normal LP type despit hyperlipidaemia at screening.
[3] II A, II B III, IV and V indicates type of hyperlipoproteinaemia (4).

of HLP subjects into different types of HLP is illustrated in Table III with one lower and one higher set of values. As expected, the higher the cut off point the more sera were classified as N. The most dramatic effect of going from low to high cut off points was in both sexes a pronounced reduction of the number of type II B. For type II A there was a sex difference so that the number of males was only reduced by 16 per cent going from the lowest to the highest cut off value while females were diminished by nearly 40 per cent. The number of subjects classified as having type IV HLP was slightly raised with increasing cut off points. The considerable differences in the number of subjects allocated into different types of HLP especially type II B HLP when moderate changes were made in the cut off points demonstrate the arbitrary nature of the typing system.

Serum lipoprotein concentrations in the different types of hyperlipoproteinaemia

The individuals of each sex in the interspective sample were allocated into 6 different groups according to type of HLP.

The distribution of the LP concentrations (Fig. 6–11) particularly LDL and VLDL, were strongly skewed which is partly a result of the fact

Table IV Mean, median and range of concentrations of cholesterol (chol, mg/100 ml) and triglycerides (TG mmol/l) in the different LP fractions of males with different types of HLP and in controls (C). N = normal type of HLP (in spite of HL at screening), n = number in each group. Chol/TG = mg/100 ml/mmol/l

Group	Estimate	Lipoproteins VLDL chol	VLDL TG	VLDL chol/TG	LDL chol	LDL TG	HDL chol	HDL TG
C n=46	Mean	12	44	27	125	.32	62	15
	Median	12	.37	–	125	.31	58	.15
	Range	4–30	.10 1.27	16–60	79–189	17–.53	38–97	.09–.27
N n=35	Mean	20	.80	25	178	45	57	.21
	Median	20	90	–	183	46	53	.21
	Range	5–35	.27–1.22	14–37	99–218	.26–70	32–118	.09–.52
II A n=31	Mean	24	77	32	269	.69	51	.21
	Median	22	.82	–	260	70	51	.20
	Range	9–71	.27–1.22	17–91	221–417	.41–1.30	30–75	.06–.37
II B n=14	Mean	43	1.87	24	239	75	45	.25
	Median	43	1 62	–	237	74	45	.25
	Range	30–63	1.29–3.20	18–36	223–261	.34–1.07	32–58	16–.31
III n=11	Mean	99	2.95	37	139	.64	42	.30
	Median	84	1.81	–	133	.60	41	.26
	Range	47–287	1.54–10.25	26–52	68–205	.29–1.37	35–54	.22–.50
IV n=94	Mean	65	3.66	21	156	61	43	.30
	Median	54	2 68	–	162	.58	43	.28
	Range	22–207	1.27–21.53	8–38	63–219	.29–1.09	25–69	.06–79
V n=3	Mean	190	26.75	12	62	.64	34	.57
	Median	185	10.15	–	60	72	35	.56
	Range	66–319	5.18–64 92	5–18	49–76	.39–.81	22–45	.43–72

See text, p. 20.

Table V† Mean median and range of concentrations of cholesterol (chol, mg/100 ml) and triglycerides *(TG mmol/l) in the different LP fractions of females with different types of HLP and in controls (C).* See legend to Table IV

Group	Estimate	Lipoproteins VLDL chol	TG	chol/TG	LDL chol	TG	HDL chol	TG
C n=61	Mean	13	46	28	131	.37	67	18
	Median	12	.37	–	129	.35	64	16
	Range	2–36	13–1 38	12–58	79–207	.20–67	36–102	.07–.61
N n=31	Mean	18	68	27	189	.53	66	.23
	Median	17	67	–	193	.52	63	.23
	Range	4–40	15–1.21	15–52	100–219	.23–.81	40–113	10–.35
II A n=46	Mean	22	67	37	262	.64	66	.26
	Median	22	66	–	249	.62	63	.25
	Range	7–40	18–1 19	20–58	222–412	.32–1.00	45–101	.14–44
II B n=12	Mean	44	1.75	25	253	.89	50	.26
	Median	42	1 68	–	261	.88	48	.26
	Range	33–72	1.31–2.68	20–35	220–288	.65–1.41	32–74	17–40
III n=6	Mean	71	1.76	40	182	.83	45	.26
	Median	62	1.74	–	184	.89	44	.26
	Range	44–121	1.26–2.50	30–68	125–228	.50–1.08	38–58	18–.36
IV n=30	Mean	51	2.45	22	171	74	50	.33
	Median	44	2.06	–	179	70	48	.29
	Range	24–102	1.30–5.46	12–33	83–213	.37–1.53	32–85	17–85
V n=1		128	15.17	8	81	79	37	72

† See text, p. 20.

that cut off points have been used fo these LP concentrations in the formation of the groups. Therefore only mean, median, minimum and maximum concentrations for cholesterol and TG of the LPs in the different groups are given in Tables IV and V In the control group which originally was defined as based upon total serum lipids, all subjects except one male (VLDL TG of 1 27 mmol/l) and on one female (VLDL TG of 1 38 mmol/l) had values for VLDL TG and LDL cholesterol which were below the upper normal limits used for these LPs.

VLDL concentrations. The distributions of the VLDL cholesterol and TG concentrations in the different groups are given in Figures 6 and 7 (Tables IV and V). By definition two groups – N and II A – had VLDL TG below 1 25 mmol/l N and II A were similar and differed from the control groups by having a greater frequency of higher values for VLDL TG and considerably higher mean/median values for both VLDL cholesterol and TG.

The three types having elevated VLDL TG had different distributions of this LP lipid. Type II B had the narrowest curve and the lowest mean/median value *Type IV had the broadest distribution* while type III was intermediate

The distribution curves for VLDL cholesterol in the different types paralleled in essence those of VLDL TG concentrations except for type III. Thus, there were relatively low VLDL cholesterol concentrations in controls, N and type II A HLP and higher values in type II B III IV and V HLP In contrast to the distribution of VLDL TG the VLDL cholesterol showed a greater frequency of

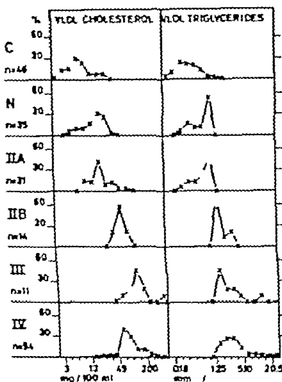


Fig. 6. Distributions of VLDL cholesterol and TG concentration in logarithmic scales in males in the different types of *HLP*. C control group N normal LP type despite hyperlipidaemia at screening (see text). II A, II B, III and IV indicat type of HLP. The line indicates the cut off point used for defining VLDL TG in typing of HLP.

high value in type III than in type IV. Also both mean and median values for VLDL cholesterol were greater in type III than in type IV while the opposite was true for VLDL TG. Therefore the highest ratio cholesterol/TG in VLDL was seen in type III HLP (Table IV and V). In both sexes also some subjects with HLP of type II A and N had relatively high concentrations of VLDL holesterol in relation to the TG content resulting n a high holesterol/TG also in VLDL.

Men tended to hav higher VLDL TG and cholesterol concentrations than women in all HLP groups. This was most clearly seen in types III nd IV.

LDL concentrations (Fig. 8 and 9. Table IV and V). By definition, types N nd IV had LDL cholesterol below 220 mg/100 ml nd types II A and II B had values above this cut off point. For type III no criteria was applied with regard to LDL cholesterol, but only one – a female – of the 17 subjects with type III HLP had a LDL cholesterol above ?0 mg/100 ml. As in the case of VLDL TG concentrations, there was a difference between controls and N with higher mean and median values for LDL cholesterol in the N groups. In both sexes th distribution of *LDL* cholesterol in type II A and type II B was different, type II A having broad and II B a steep and narrow distribution of LDL cholesterol.

In men low LDL cholesterol concentrations (below 100 mg/100 ml) (17A) were seen in 11 per cent of type IV subjects 17 per cent of type III subjects and all 3 type V subjects compared to 2? per cent in controls. All these three types of HLP characterized by VLDL TG elevation had minimum values of LDL cholesterol below that of the

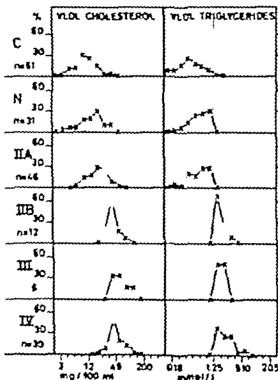
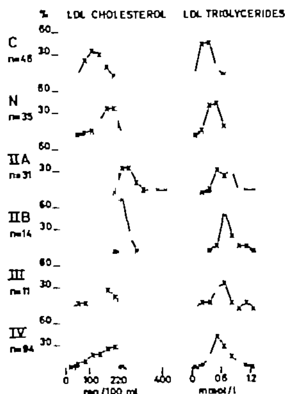


Fig. 7. Distributions of VLDL cholesterol and TG concentrations in logarithmic scales in females i the different types of HLP. See legend to Fig. 6.



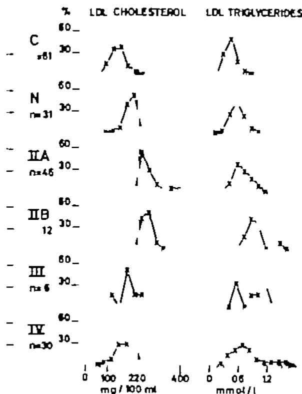


Fig. 8. Distributions of LDL cholesterol and TG in males in the different types f HLP The line indicates the cut off point used for defining LDL cholesterol in typing f HLP Symbols, see legend to Fig. 6.

Fig. 9 Distributions of LDL cholesterol and TG in females in the different types of HLP See legend to Figs. 6 and 8.

controls. The only woman with type V HLP had low LDL cholesterol concentrations, otherwise low values for this LP lipid were seen only occasionally in women. In control women it was found in 15 per cent.

The LDL TG concentration was higher than that of the controls in all types of HLP thus also in types characterized by "normal" LDL cholesterol concentrations i. e. N III IV and V Therefore in contrast to VLDL, th situation of the distribution curves of LDL TG in the different types did not parallel those of LDL cholesterol. The highest mean LDL TG co centration was found in type II B where it was more than twice that of the control group.

LDL cholesterol and TG concentrations were slightly higher in women than in men in all types but type II A.

HDL concentrations (Fig. 10 and 11 T bles IV and V) Although HDL levels are not considered in the definition of the different types of HLP several interesting differences were found between the different types HDL cholesterol concentrations were lower in all HLP types than in the control groups. This was most pronounced for the types with an elevation of VLDL TG concentration. In these types mean HDL cholesterol was in women in no instance above 50 mg/100 ml while in the other groups it was above 65 mg/100 ml. In men the corresponding figures were 45 and 50 mg/100 ml. The highest individual values for HDL cholesterol concentrations were seen in the N types. Mean HDL cholesterol concentrations were higher in women than in men in all types.

In contrast to HDL cholesterol concentrations, HDL TG was higher in the HLP types with raised

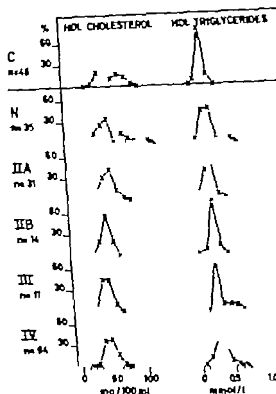


Fig. 10. Distributions of HDL cholesterol and TG in males in the different types of HLP. Symbols, see legend to Fig. 6.

VLDL TG. The highest values for HDL TG were seen in type V. In both type IV and V values close to 1 mmol/l occurred in contrast to the mean value of 0.15 mmol/l in the control groups.

Lipoprotein lipid composition

The frequency distribution curves of cholesterol and TG in the LP classes indicated that in some types of HLP the relation between cholesterol and TG was different from that in other types. A LP class may consist of LP molecules of identical or different kinds. Within each kind of LP molecule the proportion of among other things cholesterol/TG is the same. If changes in the concentration of a LP class consisting of identical LP molecules were due to changes in the number of these molecules, the composition of the LP class such as e. g. the relation between cholesterol and TG would therefore remain constant. The same would hold true if a LP class normally containing different kinds of LP molecules underwent a change in the number of molecules but with a retention of a constant ratio between the numbers of the different LP molecules. If however changes in the concentration were particularly due to increases/decreases in certain LP molecules with a different composition with regard to the ratio cholesterol/TG such concentration changes would be accompanied by alterations and disappearances in the constant relation between cholesterol and TG.

In order to analyze further the composition of the LP classes in the various types of HLP we considered all HLP as a one population, defined by the presence of HLP and performed correlation (Table VI) and regression analysis (Fig. 12–14) on the whole sample of HLP subjects for each LP class. The regression equation, if significant was

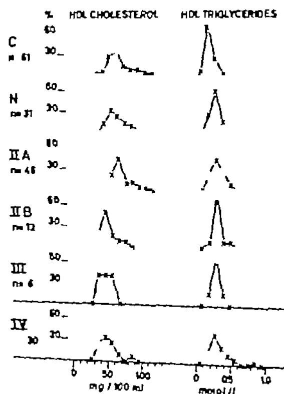


Fig. 11. Distribution of HDL cholesterol and TG in females in the different types of HLP. Symbols, see legend to Fig. 6.

then regarded as a curve describing the average value for the "dependent" variable – cholesterol – in relation to the "independent" – TG From the inspection of Figures 12–14 one could then evaluate whether all types of HLP had the same relation between cholesterol and TG in a LP class, i e were distributed equally on both sides of the least squares curve.

VLDL composition. For VLDL (Fig. 12 Table VI and VII) there was a close positive correlation between its content of cholesterol and TG. For practical purposes, the relation is given with logarithmic values Both linear and quadratic regressions were employed and the quadratic term was significant ($p<0.01$) for both males and females. When non-logarithmic values were used the regression equation contained the origin. There was no difference in the relationship between male and female VLDL. All types of HLP except type III fitted into the same relation between cholesterol and TG For type III the cholesterol content was consistently higher in relation to the TG content than the average relation would have predicted. However a few type II A and some types IV HLP *had a similar high cholesterol content in relation* to the TG content in VLDL. Exclusion of type III

Table VI. Correlation coefficients between LP lipids

	^{e}log VLDL TG	LDL cholesterol	LDL TG	HDL cholesterol	HDL TG
MALES 135					
^{e}log VLDL cholesterol	90	– 75	.09	– 47	.55**
^{e}log VLDL TG		– 77	.01	– 46	.58**
LDL cholesterol			.21	42***	–.54
LDL TG				– 10	22**
HDL cholesterol					–.093
FEMALES n 84					
^{e}log VLDL cholesterol	93	–.59	.52 **	–.60 **	45 **
^{e}log VLDL TG		– 65 **	40*	–.59	49 **
LDL cholesterol			– 044	.37	–.26
LDL TG				– 26	.38
HDL cholesterol					011

and mdxat significant correlations on the 5 1 and 0 1 percent level respectively

Tabl VII Equations fo the dependence of different LPs on ^{e}log VLDL TG in mmol/l (x) in subjects with HLP

y	MALES Equation	P		FEMALES Equation	P	2
^{e}log VLDL cholesterol mg/100 ml	y 3.343 0.774 – 0.00416 2	<0.001	<0.02	y 3.365 0.791 x – 0.083 2	<0 001	<0.005
LDL cholesterol mg/100 ml	y 231 – 62.0	<0.001	–	y 233 – 48.9 x	<0.001	–
LDL TG mmol/l		>0 05	–	y 0.71 0.126	<0.001	–
HDL cholesterol mg/100 ml	y 49 – 5.4	<0.001	–	y 58 – 11 7 x	<0.001	–
HDL TG mmol/l	y 0.22 0.046 +0 020 2	<0.005	<0.001	y = 0.26 0 057 x + 0 037 2	<0.001	<0

Both linear and quadr tic regression was pplied. The former is given when the quadratic term was not significant.

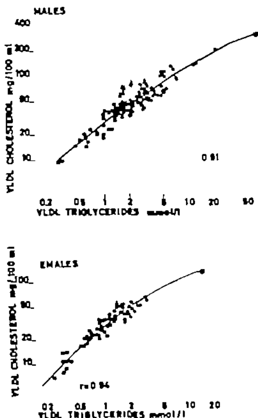


Fig. 12. VLDL cholesterol in relation to VLDL TG in males and females with HLP. VLDL cholesterol and TG are given in log scales. The lines are the regression lines of log VLDL cholesterol on log VLDL TG. Symbols as in Fig. 3.

from the correlation analysis did not however effect r values of the regression equations probably due to the low number of type III HLP.

Thus, it appears that in types II A, II B, IV and V we have VLDL LP with the same relation between cholesterol and TG suggesting that the *level of VLDL is directly determined by the* number of similar VLDL particles. It has to be considered that the relation was not linear but rather of a parabolic nature so that at higher VLDL TG there was proportionally less cholesterol. This might be explained if the VLDL particles increased in size with rising VLDL concentrations as has been reported (49). This would imply decrease of surface (rich in free cholesterol and protein) to volume (TG rich). In type III HLP the VLDL particles are more cholesterol rich, which has previously been described (29).

LDL composition. For LDL the relation of cholesterol to TG was positive but weak for males and insignificant for females. Figure 13 shows that there were several differences between the types of HLP in both males and females for this relation. Type V had a very low LDL cholesterol in relation to its LDL TG concentration. Type III had also a consistently low cholesterol concentration in relation to its TG content but type V was further below the line than type III. Type II B and even more type II A had high LDL cholesterol in relation to LDL TG. Some type II A who clinically had tendon xanthomata which have been called type II A–X (14) had a particularly high cholesterol *content in relation to the TG content.*

Figure 13 suggests that there was a strong positive linear relationship between the cholesterol

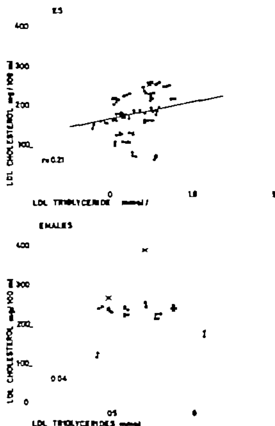


Fig. 13. LDL cholesterol in relation to LDL TG in males and females with HLP. The line is the regression line of LDL cholesterol on LDL TG (only significant in males). Symbols as in Fig. 3. x indicates II A-X (see text).

and TG concentrations of LDL for type III HLP The existence of such a relation was studied by linear regression analysis separately for the various types. There were no major differences between males and females. The r-values for the relation between LDL cholesterol and TG were (both sexes pooled) for types II A, II A–X, II B III and IV 0 17 0 52, 0.37 0 83 (p<0 001) and 0.02 respectively

The weak or non-existing relationship between cholesterol and TG in LDL shows that this LP class was not composed of one species of well defined LP molecules in the entire HLP population. There was a clear tendency however for all types except type IV to show a fairly characteristic LDL composition with either high or low cholesterol content in relation to the TG content. Our LDL LP is defined as having a density range of 1 006–1 063 Within this range there are at least two different LP classes of different composition, LDL_1 (d=1 006–1 019 S_f12–20) and LDL_2 (d=1 019–1 063 S_f 0–12) the former being more TG rich, the latter more cholesterol rich. It is thus possible that the two extremes with regard to LDL composition, type V and type II A X had mainly LDL_1 respectively LDL_2 in the isolated LDL. Gofman and co-workers in their early work with the analytical ultracentrifuge showed that patients with tendinous xanthomata indeed had a great crease of the S_f 0–12 LP (26).

Type III HLP the diagnosis of which was based n two VLDL criteria only also showed a characteristic LDL composition with LDL rich in TG in relation to its cholesterol content. Furthermore LDL from type III HLP was the only LDL fraction having a significant correlation between its TG and cholesterol content (r = 0.83) suggesting that type III LDL is the most homogeneous LDL LP at least with regard to the cholesterol/TG rel tion.

HDL composition. For HDL (Fig. 14) there were no significant correlation between its cholesterol and TG content for the whole HLP population. Only for type III was there a correlation r = 0 81 for men. Type V was characterized by a low cholesterol and high TG content Females with type II A had a higher cholesterol content than the types with VLDL elevation in relation to the TG concentration. This wa not so pparent fo males.

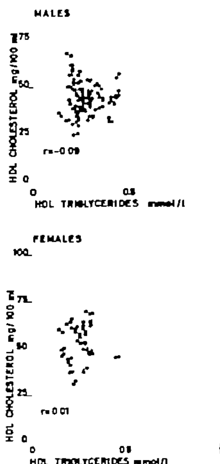


Fig. 14. HDL cholesterol in relation to HDL TG in males and females with HLP Symbols as in Fig. 3.

Some caution must be exercised in the interpretation of these data as the concentration of HDL TG is very low and thus subject to analytical errors. Furthermore there may be som uncertainty with regard to th specificity of the TG method in this fraction as th HDL LP was not isolated from the other serum constituents. However the lack of correlation between HDL cholesterol and TG suggests heterogeneity of this LP except for type III HLP

Continuous relation fo lipoprotein classes between types of hyperlipoproteinaemia

VLDL and LDL are the two classes of LP used in the classification of HLP Since TG is the major component of VLDL and cholesterol that of LDL,

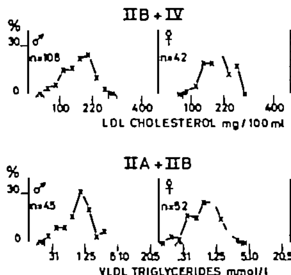


Fig. 15. Distribution of LDL cholesterol in males and females with elevated VLDL TG concentration (above) and of VLDL TG in males and females with elevation of LDL cholesterol levels (below).

VLDL TG nd LDL cholesterol have been used for the classification. When VLDL is "elevated this HLP is further subdivided into type IV with normal LDL and type II B with raised LDL. However the distribution curves for LDL cholesterol were skewed to the left and to the right respectively for these two types, and in fact when they were pooled the common LDL cholesterol distribution curve (Fig. 15) for HLP with elevation of normal (pre-β moving) VLDL did not indicate the presence of two LDL populations. On the contrary the LDL distribution curve was continuous and fairly normal. A similar picture evolves if we consider instead the HLPs with "raised LDL cholesterol They are also subclassified into two types, II A with "normal" VLDL and II B with raised VLDL. If we look at the distribution curves f VLDL TG in these two types of HLP they were kewed to th left and right respec tively When they were pooled th common distribution curv for VLDL TG for all HLPs with raised LDL cholesterol (Fig. 15) did not show any significant signs of bimodality but was fairly con ti uous.

From these fairly continuous distribution curves the cut off points used for both LDL and VLDL seem arbitrary and perhaps even artificial. Furthermore, it was clear from the description of the different types of HLP that some LP characteristics, for example low HDL cholesterol were related more to a certain LP abnormality (VLDL TG elevation) than to a specific type of HLP

These characteristics suggest the existence of continuous relations between the various LP classes in this material of HLP Since the classification of HLP into rigid types breaks any such relations, as we have discussed previously (?0) we have analyzed the presence of such continuous relations between VLDL and the other LP classes in all pooled HLP subjects of each sex.

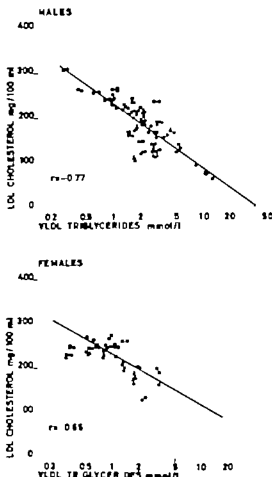


Fig. 16. LDL cholesterol in relation to VLDL TG in males and females ith HLP VLDL TG are given in log scale. The lines are the regression lines of LDL cholesterol on log VLDL TG. Symbols, see Fig. 3

Relation between different lipoproteins

Correlation coefficients between different LP lipids are given in Table VI. VLDL was fairly strongly correlated to both LDL and HDL. These relations are therefore further illustrated in Figures 16–19. Equations are given in Table VII. LDL cholesterol was negatively related to VLDL TG concentrations (Fig. 16) and the regression equations were almost identical in both sexes. Except at the highest levels of VLDL TG, LDL cholesterol could vary within rather wide limits. However, at every level of VLDL TG the *maximal* LDL cholesterol concentration seemed to be very definitely determined. Therefore only low and never high LDL cholesterol concentrations were seen with high VLDL TG levels. All types of HLP appeared to fit into this regression except II B which was above the line in both sexes. This is explained by the fact that this type by definition has an elevation of both VLDL TG and LDL cholesterol.

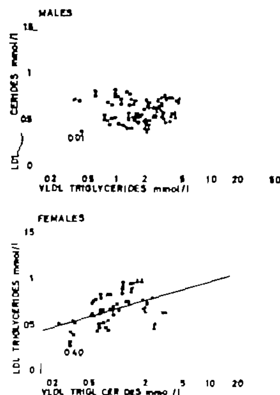


Fig. 17. LDL TG in relation to VLDL TG in males and females with HLP. VLDL TG are given in log scale. The line is the regression line of LDL TG on log VLDL TG (only significant in females). Symbols, see Fig. 3.

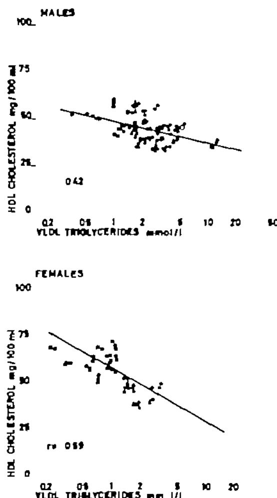


Fig. 18. HDL cholesterol in relation to VLDL TG in males and females with HLP. VLDL TG are given in log scale. The lines are the regression lines of HDL cholesterol on log VLDL TG. Symbols, see Fig. 3.

The relation between VLDL and LDL TG (Fig. 17) was positive but weak in females, and not significant in males. There did not appear to be any type specific relation.

Negative correlations existed in both sexes between VLDL TG and HDL cholesterol (Fig. 18). As a result of this, HDL cholesterol was decreased in types of HLP with VLDL TG elevation (II B, III, IV and V). All types of HLP fitted into the general relationship.

On the other hand, positive correlations existed between VLDL and HDL TG (Fig. 19). This was best described by a parabolic function with a positive quadratic term when VLDL TG was loga-

rithmic and HDL TC linear Once again the relation fitted for all types of HLP

Classificatio of hyperlipoproteinaemia based p- on serum lipid co centrations

For practical purposes it is of interest to know to what extent the determination of only serum lipids can lead t the correct diagnosis of type of HLP Thus it has been suggested to divid HL int three groups according to the concentrations of cholesterol and TG 1) elevation f only cholester ol 2) elevation of only TG nd 3) elevation of both lipids (10 19) Groups 1 and 2 have then been believed to represent type II A and IV respectively and group 3 type II B and other types such as III and IV

The value of total serum lipids in the diagnosis f types of HLP has been evaluated here by relating the values for total serum TG and cho-

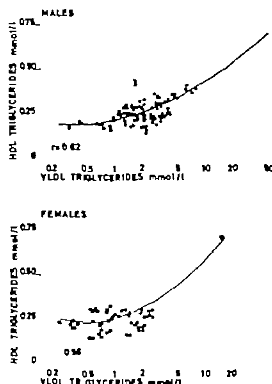


Fig 19 HDL TG i relation t VLDL TG in males and females th HLP VLDL TG are given in log scale. The lines are the regression lines of HDL TG on log VLDL TG. Symbols, see Fig. 20.

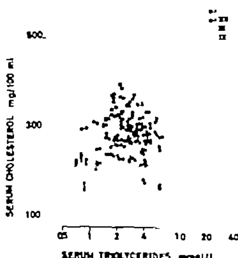


Fig. 20. Serum cholesterol and TG concentrations in men with HLP and controls at the LP determination.

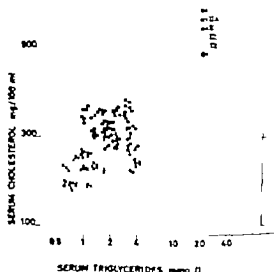


Fig. 1 Serum cholesterol and TG concentrations in women with HLP and controls at the LP determination.

lesterol to the actual type of HLP (Fig. 20 and 21). If we choose the upper limits of 320 mg/100ml for cholesterol and 2 mmol/l for TG it can be seen that lipid group 1) comprised only type II A. Group 2) comprised mainly type IV but also types II A, II B, III and V. Group 3 also comprised all types of HLP. This clearly shows how incomplete the picture is when only serum TG and cholesterol are determined. The importance and necessity of LP analysis for correct diagnosis is clearly evident.

DISCUSSION

Classification of hyperlipoproteinaemia

Five different types of HLP (type II A, II B, III, IV and V) could be identified in our sample of HL subjects. In addition, 20–25 per cent of the subjects with HL at screening had VLDL TG and LDL cholesterol concentration within normal limits at the second study and were thus classified as having a normal (N) LP pattern. This finding has probably several explanations. Both total serum cholesterol and TG concentrations tended to be lower at the second blood sampling when blood was drawn for serum LP analysis for reasons discussed above. It is likely that the decrease in total serum cholesterol and TG concentrations reflects decreases in LDL cholesterol and TG as well as in VLDL TG and cholesterol levels. Thus, in a number of subjects these concentrations could have decreased beneath the cut off points for HLP. However, in addition as seen in Figures 10 and 11 the subjects with the highest HDL cholesterol concentrations were found in the N groups. As HDL cholesterol is part of total serum cholesterol it is possible that some subjects already at the first blood sampling had hypercholesterolaemia due to hyper HDL (α)-cholesterolaemia with LDL cholesterol concentrations within normal limits. Also in the typing of HLP, small changes in the cut off points for VLDL TG and LDL cholesterol greatly affect the allocation of subjects with hyperlipidaemia into different types of HLP. Thus, as illustrated in Table III, changing the cut off points for VLDL TG and LDL cholesterol from 1.10 mmol/l and 200 mg/100 ml to 1.40 mmol/l and 240 mg/100 ml doubled to trebled the number of subjects with "normal" LP pattern.

Advantages and disadvantages of the present system for classification of hyperlipoproteinaemia

The present typing system for HLP was suggested by Fredrickson, Lees and Levy (24) and has been slightly modified (4). It has several advantages. One major advantage is that it stimulates thinking in terms of LP instead of total lipid concentrations. Another is its simplicity in the sense that it facilitates communication. Indeed, it is easier to say and write type II B instead of "hyper VLDL-lipoproteinaemia combined with hyper LDL-lipoproteinaemia".

However, the typing system also has several drawbacks. It has been argued that individuals often oscillate between several types of HLP within a short time. Also, one may easily become inclined to look erroneously at the types of HLP as separate disease entities. An example of this attitude is the expression "type II B disease". However, it has to be borne in mind that the typing system has nothing to do with etiology and pathogenesis and that the typing is done on the plasma sample and not on the patient (four P problem, 13).

In the present study some other disadvantages of the typing system were evident.

1. By necessity the cut off points must be arbitrarily defined as the distribution curves of the LPs are unimodal and continuous.
2. The allocation of subjects into different types of HLP was highly dependent on the cut off points chosen, not only in the sense that increasing or decreasing the cut off points altered the number of subjects having HLP but also in that the relative frequencies of the various types changed. (Table III)
3. From Figure 15 it was evident that looking at all subjects with elevation of LDL cholesterol or VLDL TG, an approximately "normal" distribution is achieved with the other diagnostic LP. For example, VLDL TG in type II A and II B formed together a "normal" continuous relation.
4. A continuous relation existed between LDL cholesterol and VLDL TG in the HLP popula-

tion (Fig. 16). This smooth relation is indeed completely interrupted by the use of cut off points for these LP lipids and misleadingly transforms the continuous relation into discrete populations.

These findings make the typing system p- pear highly arbitrary even artificial and sometimes misleading.

Metabolic aspects on lipoprotein composition and lipoprotein interrelations in the different types of hyperlipoproteinemias

The metabolic functions of serum LP is to transport fats between tissues. One major rout in the fasting state is transport of TG synthesized from fat or carbohydrates, from the liver to peripheral tissues for oxidation and storage (47) Th LP metabolism during this transport is schematically depict d in Figure 22. In essence the TG are synthesized in the liver and incorporated into VLDL, which are secreted from the liver into blood. In blood they pick up apolipoproteins from HDL (5). The TG rich VLDL is then at tacked by the enzyme syst m lipoprotein lipase which splits off f tty acids from the TG. By this process VLDL loses more and more TG and eventually becomes degraded to LDL_2 LDL_2 the final stage of this TG transport is the catabolised. The details of these processes are not known. It is believed that the first step when VLDL is attack d by lipoprotein lipase a d loses TG leads to the f rmation of intermediary par ticles" (45) which normally are metabolized rapidly to LDL by for instance lipase activities. During these processes apolipoprotein leaves the less and less TG rich LP and is transferred to HDL (23).

We want to briefly discuss our major findings of LP concentrations, LP composition and LP inter relations against this schematic metabolic back ground assuming first orde kinetics to exist at all levels.

Type II A

Type II A defined as raised LDL holesterol, had a LDL characterized by high cholest rol content in relation to TG content. This is probably due to accumulation of LDL_2 th final product in the

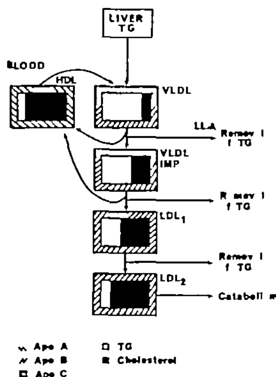


Fig. 22. Schematic picture of the metabolic interrelation of VLDL, LDL and HDL in the transport of TG from the liver. VLDL – IMP VLDL-intermediary particles, LLA lipoprotein lipase activity Apo A, B, C apolipoprotein A, B, C.

long chain of events in VLDL LDL metabolism. The most obvious kinetic explanation for rise i only this LP class would be a specific block in th catabolism of LDL_2 In fact studies with LDL labelled in the protei moiety with ^{125}I have shown that the fractional LDL catabolism is reduced in type II A–X patients compared to or mal subjects (32) In accordance with this concept of a simple defect in catabolism of LDL_2 there were no general bnormalities in HDL or VLDL in the groups with type II A. However som subjects tend d to have increased cholesterol/TG ratio in VLDL. This m ght suggest a d fect in the handling of VLDL-intermediary particles (see below under type III)

Type II B

Type II B defined by raised LDL cholesterol and VLDL TG had compared t type II A a LDL

characterized by a higher TG content in relation to its cholesterol content. In fact II B had the highest LDL TG This suggests that type II B had an accumulation not only of LDL_2 but also of the TG rich LDL_1 A combination of raised VLDL, LDL_1 and LDL_2 is from the kinetic point of view most easily explained by an increased production by the liver of VLDL which would increase the turnover and concentration of these LP classes. The changes seen in HDL in this type are characteristic for the elevation of VLDL and will be discussed when the relation between VLDL and HDL is discussed.

Type III

Type III defined by raised VLDL TG and presence of a β-VLDL" which is believed to represent the VLDL-intermediary particle (45) had a TG rich LDL which over its entire range of concentration has a strong correlation between its cholesterol/TG content. This LDL composition may fit with the accumulation of a TG rich LDL_1 of fairly constant composition which may be closely related to the VLDL intermediary particles. In fact Gofman et al demonstrated that patients with xanthoma tuberosum who thus probably had type III HLP had raised S_f 12–20 (LDL_1) and 20– (VLDL) LP (26) The rise in not only VLDL TG also in the ratio cholesterol/TG in VLDL ly suggests the accumulation of VLDL-inter ury particles. The metabolic block in type III would then lie in the conversions of VLDL-intermediary and LDL leading to accumulation of these LPs.

Type IV

Type IV defined by elevation of VLDL TG showed a wide range of LDL cholesterol and TG concentrations and also a wide range in the relation between these two LDL lip ds. This suggests a great variatio in the relation between LDL_1 and LDL_2 in this HLP A simple explanation for the rise in VLDL TG in type IV would be a block in the catabolism of VLDL. All other things being unchanged this would not ffect th others LPs. In fact several type IV HLP had fairly normal concentrations nd compositio of LDL. However many also had low to very low LDL cholesterol and high LDL TG in relation to the cholesterol cont nt thus suggesting low LDL_2 The negative relation between VLDL TG and LDL cholesterol suggests that LDL_2 decreased particularly at high VLDL TG. There are two main possible reasons for a low LDL_2 1) Increased fractional catabolism of LDL_2 2) Decreased formation of LDL_2 At least two mechanisms seem possible for a decreased formation. The first is a decreased production (turnover) of VLDL TG e. g. by feed-back inhibition of secretion from th liver by the high VLDL levels. The second is a decreased formation of LDL_2 at a normal VLDL TG turnover This may occur if the VLDL particles were larger than normal thus having a decreased surface/volume ratio. As the hydrophobic TG probably are in the interior and the hydrophilic B protein and the free cholesterol on the surface large VLDL would imply lower content of B protein the protein precursor of LDL, per unit of TG mass. Thus for the transport (turnover) of the same amount of TG less B protein and thus less LDL_2 would be formed.

It is also possible that in some type IV where both LDL cholesterol and TG were high – although not elevated – the kinetic mechanism for the HLP was an increased formation of VLDL as is suggested for type II B

The pathogenesis of type IV HLP has not been elucidated Some studies indicate that the major metabolic defect is reduced catabolism of VLDL (8 28) while others favour an increase in VLDL prod ction (1 46) It is quite possible that these discrepancies in part may be due to a considerable ***heterogeneity*** of type IV HLP with various pathogenetic mech nisms behind the increase in VLDL TG It is possible that consideration of LDL concentration and composition as discussed above may help to distinguish between type IV HLP due to either defect catabolism of or to increased synthesis of VLDL.

Type V

Type V defined by the presence of chylomicrons in fasting serum and raised VLDL, had a LDL characterized by very low cholesterol concentration and a high TG content in relation to the

cholesterol content, suggesting very low LDL_2 levels. It is believed that chylomicrons and VLDL are at least initially catabolized by the same mechanisms involving lipoprotein lipase activity (47). The most simple mechanism for this HLP from the kinetic point of view would be a block in the first step of VLDL and chylomicron degradation. It is possible that the catabolic block in VLDL and chylomicron metabolism is more severe than in type IV The probably very low LDL_2 levels may have the same two e planations as discussed above for type IV Both in type IV and type V turnover studies are needed to resolve these problems.

The postulated decrease in catabolism of VLDL and chylomicrons may both in type *IV* and V be due to a decrease in lipoprotein lipase activities (7 44) or to reduced fatty acid incorporation into adipose tissue (FIAT) function (21).

Lipoprot in int rrelationships in hyperlipoprot inaemia

Both LDL and HDL showed strong interrelations to VLDL. In LDL this was particularly true for LDL cholesterol showing a negative correlation while LDL TG had no or a weak, positive correlation. This suggests from the compositional aspects discussed above that LDL_2 was strongly negatively correlated to VLDL. We know that at the beginning of this relation (Fig. 16) with high LDL cholesterol a d normal VLDL TG (II A) the fractional catabolism of LDL is decreased (3) One simple explanation for the decl ne in LDL cholesterol with rising VLDL would be that whe VLDL TG increases there is first a normalization and then an increase in the fractional catabolism of LDL. In f ct when moving along this line from type II A, one first encounters type II B and types we assumed a *normal* LDL catabolism (nd raised VLDL turnover) in th discussion bove. Further on along the lin w encou t r the severe type IV and type V f which types w have already discussed the possibility of n increased fractional catabolism f LDL. To summariz th simplest explanation for the negat ve correlation between VLDL TG and LDL cholesterol would be contiuous increase in LDL fractional catabolism from reduced via normal to increased values with rising VLDL TG This hypothesis needs turnove studies to be validated. Needless to say there are other but more complex possible mechanisms.

VLDL had different relations to the two HDL lipids. There existed a positive relation to HDL TG. A similar relation was found by Lindgren et al. (36) in 16 healthy subjects. The role of HDL in TG transport is far from clear However HDL TG increases during alimentary lipaemia (38) Further more, during in vitro incubation of human serum, TG is transferred form VLDL to HDL in exchange fo cholesterol esters (39).

HDL cholesterol, on the other hand, had a negative correlation to VLDL TG. A negative correlation between serum TG and HDL cholester *ol has bee reported previously* (*11*). *The* cause of this negative relation is not known. Some of the HDL may be combined t VLDL (25) (Fig. 22) nd thus t high VLDL levels the HDL concentra tion may decrease. It is also possible that l w HDL cholest rol may be a primary f ct r in the HLPs with VLDL elevation. At least one component of th HDL system, polipoprotein C is of impor tance f VLDL cat bolism as it is an activator of the lipoprotein lipase activity (33) Furthermore HDL is mportant in the LCAT reaction (41) which possibly also is invol ed in the cat bolism f VLDL (41 51)

SUMMARY

1 In order to study lipoprotein (LP) abnormalities in asymptomatic subjects with hyperl pidaemia, serum cholesterol and triglycerides (TG) were determined in bout 70 000 active professional men and women attending health ontrol linked to their employment.

2 Quantitative LP analysis w th prepar tive ultracentrifugation, separating ery low (VLDL) low (LDL) and high (HDL) density LP as subsequently performed on th sera f 314 asymptomatic subjects, who at th screening had serum cholesterol ≥350 mg/100 ml and or TG>1 mmol/L. Cholesterol and TG concentrations ere determined in each of th LP classes Paper electrophoresis was run on whole serum and on the top and bott m fractions after separation in ultracentrifuge at d=1 006. Typ

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